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REVIEW

Tumour-derived miRNAs and bone metastasis

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Skeletal metastases are complications of epithelial cancers, among which breast, prostate and lung carcinomas are the most osteotropic. In primary tumours, a subset of cancer cells undergoes epithelial–mesenchymal transition, acquires mobility to migrate into the surrounding stroma and seeds at distant sites to grow. The specific development of bone metastasis requires the recruitment of circulating tumour cells in the bone marrow, their adaptation to survive in the surrounding microenvironment where they alter the functions of osteoclasts and osteoblasts, and hijack signals coming from the bone matrix. Each of the molecular pathways underlining these steps is regulated by multiple factors, through the tight control of genes expressed by cancer cells interacting with cells from the bone microenvironment. In this context, miRNAs can act as master regulators of gene expression to control multiple aspects of bone metastasis formation, including cancer cell escape from the primary tumour site, cancer cell dissemination to bone and invasion of the bone marrow, as well as secondary outgrowth and tumour–stroma cell interactions. In the clinic, specific miRNA signatures have been identified in osteotropic cancer cells, raising the possibility that miRNAs could be used as biomarkers of bone metastasis. The regulatory activity of miRNAs in the bone microenvironment also suggests that miRNAs could be promising therapeutic targets.

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Introduction

Breast, prostate and lung primary carcinomas are the most common osteotropic tumours that spread to skeleton with strong affinity at late-stage disease. Less frequently, bone metastases are also diagnosed in thyroid, kidney, bladder and colon carcinomas. Breast and lung carcinomas result in mainly osteolytic bone destruction due to enhanced osteoclast activity whereas prostate cancer patients have predominantly osteoblastic lesions, characterised by increased bone formation due to stimulation of osteoblast activity.[1,2] Tumour-induced dysregulation of normal bone homeostasis causes hypercalcaemia, severe pains and fractures for which only palliative therapies are proposed.[3,4] The early steps of metastasis to bone are common to most solid tumours and start with local tissue invasion and tumour cell escape from primary tumours.[5] Following tumour cell dissemination into the blood and/or lymphatic system, if the cancer cells are resistant to anoikis and escape the immune surveillance, these cells must express a specific gene signature to disseminate to bone, invade the bone marrow, adapt to the local microenvironment and finally exit the dormancy step to further colonise the tissue.[2,6,7] Each of the molecular pathways underlining these steps is regulated by multiple factors, through the tight control of genes expressed by cancer cells interacting with cells of the bone microenvironment.[8,9] Small non-coding microRNAs (miRNAs) are master regulators of gene expression which have the capacity to regulate multiple genes and thus to redirect or reprogram biological pathways.[10,11] Consequently, the dysregulation of miRNA expression in cancer cells not only impacts the intrinsic tumour cell properties and tumour-derived factors but also modulates the tumour–stromal crosstalk within primary tumour and metastatic site.[12] In this regard, miRNAs can control multiple aspects of bone metastasis including the initial steps of cancer cell escape from the primary tumour to the homing and invasion of bone, as well as secondary outgrowth and tumour–bone cell interactions[13] (**Figure 1**). A small subset of cancer cells in breast primary tumours express specific gene signatures that would be predictive of bone metastasis.[14,15] In this respect, the identification of miRNA and gene signatures expressed in primary carcinomas and their matching bone metastases would provide information on molecular mechanisms driving bone metastasis. It is very likely that before patients are diagnosed with cancer, dormant disseminated tumour cells (DTC) are already present in the bone marrow.[16] Given this information, dysregulated miRNAs that are associated with the early onset of bone metastasis might be a useful clinical biomarker of the minimal residual disease in patients with cancer. At a later stage, when skeletal destruction is occurring, miRNA dysregulation might be an attractive therapeutic target to interfere with unbalanced bone cell activity and bone resorption that prime macro-metastasis in bone.[17]

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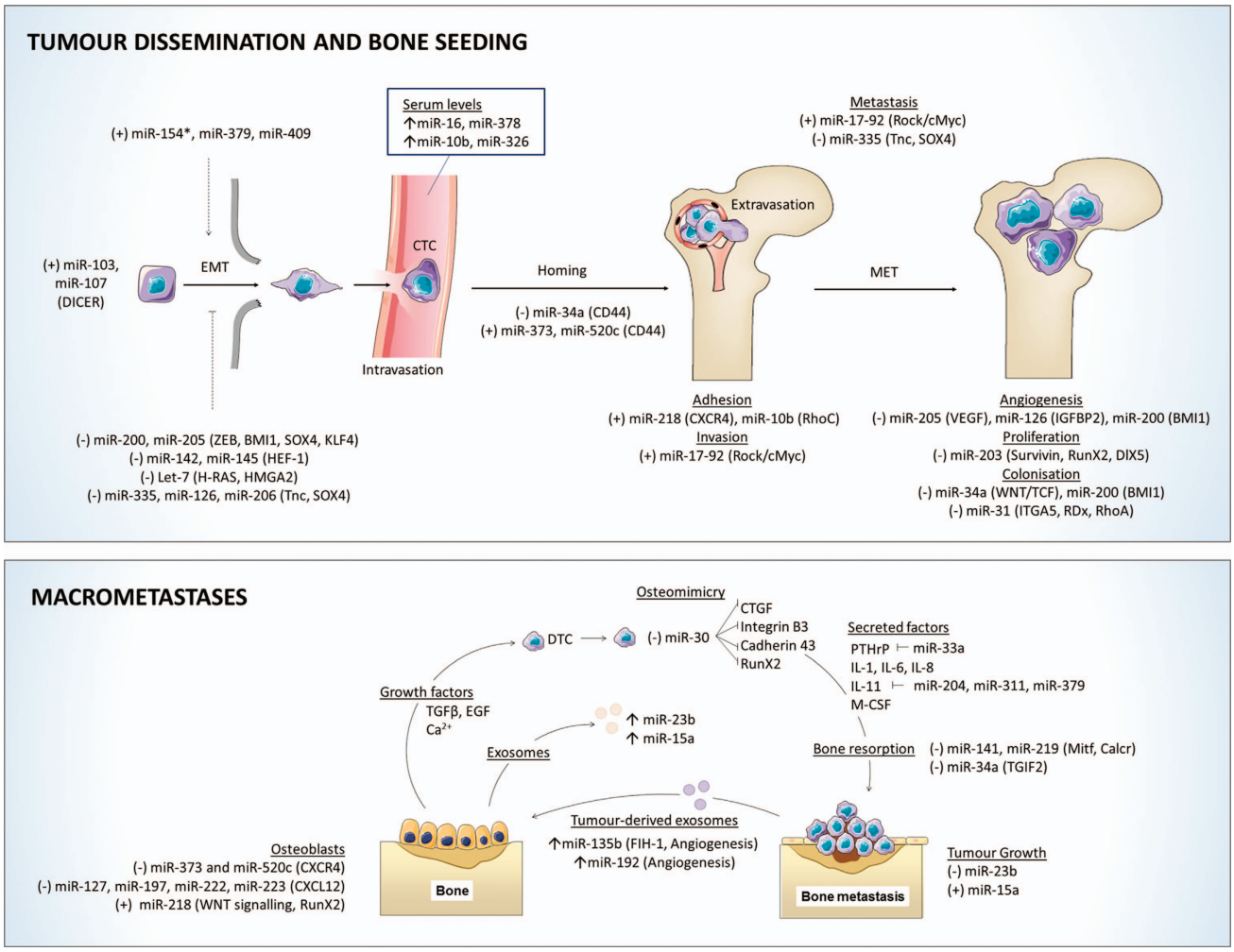

Figure 1 MicroRNAs regulate various steps of bone metastasis progression in solid tumours. MiRNAs control critical steps in cancer cell dissemination. Up- (↑) or downregulation (↓) of these miRNAs may result in the positive (+) or negative (−) regulation of gene sets and/or pathways involved in bone metastasis through the regulation of the following gene targets, indicated in brackets: BMI1, Polycomb complex protein; KLF4, Kruppel-like factor; HEF-1, Human enhancer of filamentation; HMGA2, High-mobility group AT-hook 2; Rock, Rho-associated kinase; IGFBP2, Insulin-like growth factor binding protein2; DLX5, Distal-less homeobox5; WNT, Wingless type; CTGF, Connective tissue growth factor; Mitf, bHLHe32; Calcr, Calcitonin receptor; Tnc, Tenascin C; RDX, Radixin; TGIF2, TGFβ-induced factor homeobox2; FIH-1, Factor inhibiting HIF.

In this review, we discuss how the tumour-induced dysfunction of miRNA expression controls the molecular events that mediate the metastatic dissemination of tumour cells to bone and induce bone lesions. Next, we focus on up- and downregulated miRNAs that influence the expression of genomic targets and have been experimentally validated as modulators of one or several steps of bone metastasis.

MiRNA Misexpression Alters the Intricate Steps of Bone Metastasis

Mature and active miRNAs are small, single-stranded RNAs that repress gene expression through complementary binding to the seed sequence of target mRNAs. MiRNA genes represent around 1% of the total genome across different species and are localised in intergenic or intronic regions of known genes, in the sense or antisense orientation.[10] MiRNAs are mostly transcribed by RNA polymerase II as long primary transcripts that are cleaved in the nucleus by Drosha and the DGCR8 complex to produce a 70–100-nt pre-miRNA hairpin. In the cytoplasm, an additional step mediated by the RNAse III, Dicer, generates a duplex miRNA/miRNA* which is incorporated in the RNA-induced silencing complex. One of the two strands will be selected as the guide strand whereas the other strand will be degraded. As part of this complex, mature miRNAs operate *via* sequence-specific interactions with the 3′-UTR of cognate mRNA targets leading to the destabilisation of target mRNAs and inhibition of translation.[18] Given the multigene regulatory capacity of a single or a family of miRNAs, their dysregulation has been helpful to elucidate the molecular mechanisms that govern metastatic dissemination. These endogenous regulators of gene expression are themselves subjected to regulation and are targeted by genetic and epigenetic defects.[19] Various regulatory mechanisms underline miRNA dysfunctions, including deletions, amplifications, epigenetic silencing, dysregulations of transcription factors that target specific miRNAs and the inhibition of miRNA processing. A global reduction of the expression levels of all miRNAs produced by tumours has been attributed to the downregulation in the expression of the miRNA-biogenesis enzyme Dicer.[20] Besides its involvement in

the development of primary tumours, Dicer contributes specifically to metastasis progression.[21] The profound alterations of bone homeostasis induced by *in vivo* deletion of Dicer and/or DGCR8/Ago2 in osteoprogenitor cells has suggested that tumour-secreted miRNAs might control bone cell differentiation and activity.[12] Aberrant expression of miRNAs assessed through miRNA profiling of human primary and skeletal tumours provide additional evidence that endogenous miRNAs regulate metastatic cells in primary sites and DTCs. Nonetheless, tumour-secreted factors induce specific miRNA signatures in tumour-associated stromal cells and in mature osteoclasts and its precursors during cancer-associated bone destruction.[22] Thus, miRNAs are not only critical regulators of the molecular events preceding bone homing and bone invasion but also of tumour–stromal cell interactions in the bone microenvironment (**Figure 1**).

MiRNAs in Primary Carcinomas are Potential Predictive Factors of Bone Metastasis

To detach from primary tumours, cancer cells acquire motility and invasiveness undergoing epithelial–mesenchymal transition (EMT). Then, mesenchymal cells express various factors that enable them to degrade the extracellular matrix, disconnect from the extracellular matrix scaffold and invade the local tissue, thereby facilitating intravasation of cancer cells through the basal membrane into the vasculature.[23] The EMT phenotype acquired at the primary tumour site also promotes the extravasation of circulating tumour cells from the bone marrow sinusoids to the bone marrow. When cancer cells settle down in the bone marrow, they may become quiescent for several years, even decades. Ultimately, they may exit the dormancy state and reverse the process to mesenchymal-to-epithelial transition to proliferate in bone.[24] A current concept states that a small subset of cancer cells within tumours, cancer stem cells (CSC), are tumour-initiating cells capable of self-renewal and differentiation and are very likely at the origin of metastasis.[25] MiRNAs are regulators of the early events occurring in primary carcinomas, especially the tumour-initiating abilities of CSC,[26] making them attracting predictive factors of the disease. The miR-200 family and miR-205 repress EMT and metastatic dissemination by controlling the expression of E-cadherin repressors, ZEB1 and ZEB2.[27] As an EMT repressor, miR-200c controls the tumour-initiating capacity of breast CSC by regulating the stemness-associated factors, BMI1, SOX4 and KLF4.[28] Let-7 miRNAs affect the breast CSC survival and impair metastatic progression through silencing of H-RAS and HMGA2.[29] Restoration of the tumour suppressor miR-34a in prostate CSC with enhanced tumour-initiating and metastatic properties inhibited CD44 expression and prostate cancer metastasis.[30] In the androgen-insensitive PC-3 prostate cancer cell line, ectopic expression of miR-143 and miR-145 prevented EMT, reducing the migratory capacities of these cells *in vitro* and their propensity to metastasise to bone *in vivo*.[31] Further, miR-145 attenuates EMT and invasion of PC-3 cells through repression of the oncogenic protein HEF1.[32] The low miR-143 and miR-145 expression levels in prostate tumours and in bone metastasis biopsies were negatively correlated to bone relapses, free prostate-specific antigen level and the Gleason score, suggesting that these miRNAs could be used as biomarkers to discriminate different stages of prostate carcinomas and predict bone metastasis relapse. In contrast, miR-154*, miR-379 and miR-409-3p/-5p promote tumourigenesis, EMT and bone metastasis of human prostate cancer.[33] Dicer is a target for miR-103/107, whose expression is associated with metastasis and poor outcome in breast cancer patients.[21] This miRNA family attenuates miRNA biosynthesis by regulating Dicer expression and induces EMT thus enhancing the migratory ability of the cells *in vitro* and the metastatic dissemination *in vivo*.[21] Searching for general regulators of tumour spreading, Tavazoie *et al.*[34] identified a set of miRNAs (miR-335, -126, -206) that were poorly expressed in breast cancer cell lines which were highly metastatic to lung and bone. Restoration of miR-335, miR-126 or miR-206 expression reduces the lung and bone metastatic colonisation in mice. MiR-335 and miR-206 do not affect primary tumour outgrowth, but cause a reduction in cell migratory and invasive capacities. MiR-335 suppresses breast cancer cell invasion and metastasis by targeting the transcription factor SOX4 and tenascin C, a protein involved in cell adhesion. Importantly, miR-335 and miR-126 levels are lower in breast tumours from patients with relapse to distant organs (lung, bone) compared with patients who did not exhibit metastatic relapse, indicating these miRNAs function as bone metastasis suppressors and could be used as diagnostic markers. MiR-126 not only acts as a cell-autonomous metastasis suppressor but also inhibits metastatic endothelial cell recruitment and angiogenesis by coordinate targeting of IGFBP2, PITPNC and MERTK.[35] The miRNA's potential to initiate metastatic dissemination was first reported by Robert Weinberg's laboratory showing that miR-10b was highly expressed in 50% of primary breast tumours from patients who suffered metastatic relapses. Overexpression of mir-10b in non-metastatic breast tumour cell lines initiated strong invasion of orthotopically implanted mammary tumours in mice and promoted cancer cell dissemination in the lungs. Interestingly, Twist1 was identified as a transcription factor that induces miR-10b expression which, through the activation of homeobox D10 and RhoC, promoted lung and bone metastasis.[36]

MiRNAs Regulate Bone Marrow Invasion and Colonisation by Cancer Cells, and Overt Development of Skeletal Lesions

MiRNA-mediated tumour activity in bone

Circulating tumour cells expressing chemokine receptors are attracted to the bone by bone marrow-derived chemokines, leading to the homing of cancer cells in the bone marrow.[4] Once in the bone marrow, cancer cells interact with small anatomical entities, called 'niches' that line the bone surface or blood vessels. Metastatic cells target these niches where haematopoietic stem cells reside and compete for occupancy. CXCR4, the receptor of SDF-1 (also called CXCL-12), a ligand secreted by osteoblasts can be expressed by CSC to compete with haematopoietic stem cells to fit into these niches and become quiescent.[37] The interaction of CD44-expressing CSCs with hyaluronan and/or osteopontin promotes experimental bone metastasis formation.[38] Some bone-specific proteins such as bone sialoprotein, osteonectin, osteopontin and collagen also promote the homing, adhesion and subsequent colonisation of the bone marrow by tumour cells by

binding to specific cell surface receptors such as integrins αVβ3 and α2β1.[39] To identify potential metastasis-promoting miRNAs, MCF-7 breast cancer cell line transduced with a miRNA-expression library was subjected to a transwell migration assay.[40] The induction of a migratory phenotype was used to identify the miRNA-expressing vectors enriched in the MCF-7 migrating cell population. MiR-373 and miR-520c promoted cancer cell migration and invasion *in vitro* and *in vivo*. Inoculation of MCF-7 cells expressing miR-373 or miR-520c into mice induced metastatic lesions in the skull, spine and lungs. Histological analysis showed osteolytic metastases infiltrating into the medullary bone spaces and skull. CD44, which encodes a cell surface receptor for hyaluronan and behaves as metastasis suppressor in prostate and colon cancer, was identified as the direct target for miR-373 and miR-520c. Upregulation of miR-373 in clinical breast cancer metastasis samples was also found to correlate inversely with CD44 expression.[40] The c-myc-regulated miRNA-17-92 cluster is highly expressed in metastatic breast cancer and is a positive regulator of motility, invasion and metastasis. Inhibition of Rho-associated kinase signalling downregulates the miRNA-17-92 cluster and prevents breast cancer metastasis to bone in a mouse model of human breast cancer metastasis.[41] The tumour metastasis suppressor Raf kinase inhibitory protein (RKIP) represses breast tumour cell intravasation and bone metastasis in an orthotopic murine model. This occurs through the decrease of c-Myc-induced LIN28 transcription that enhances let-7 expression and inhibits its pro-invasive and pro-metastatic target genes, HMGA2 and Snai1.[42] MiRNA-10b activated by Twist1 facilitates the intravasation of breast cancer cells in the vasculature and their dissemination to lungs. Using an experimental mouse model of human breast cancer bone metastasis, we have shown that Twist1 also facilitates bone metastasis formation through a miRNA-10b-dependent mechanism.[43] Moreover, miR-10b could be a circulating marker of bone metastasis in regard of the high level of miR-10b in serum of breast cancer patients with the disease.[44] Finally, it is of interest to note that these early bone-homing steps may be reversed by acute expression of the tumour suppressor miRNA-31. Indeed, in a mouse model of bone metastasis, the induction of miRNA-31 triggered regression of already-seeded bone metastasis.[45] Further, DTC in bone marrow have to adapt to the bone environment for subsequent development as overt metastasis. This adaptive process called osteomimicry requires that tumour cells begin to express genes that are normally expressed by bone cells.[2] Analysis of primary tumour and matched metastases from breast cancer patients show that only tumour cells that are metastatic to the skeleton express proteins of bone origin (cathepsin K, osteonectin, cadherin-11, connexin-43 and Runx2).[46] Prostate cancer bone metastases express the Notch-1 receptor that induces Runx2 expression, a master regulator of osteogenesis also involved in osteomimicry.[47] Bone lesions result in the interaction between cancer cells, bone microenvironment and bone cells themselves. The tumour cells use bone-derived growth factors involved in the coupling between osteoclasts/osteoblasts to promote their own development. In turn, they secrete factors (PTHrP, interleukin (IL)-1, IL-6, IL-8, IL-11, M-CSF) that act in a paracrine manner to activate osteoclast-mediated bone resorption.[48] Cancer cells can release other growth factors that can promote (ET1, insulin-like growth factors) or inhibit (DKK1, Noggin, sclerostin) osteoblast activity.[5] These processes are accompanied by the release of growth factors and cytokines (transforming growth factor-β, insulin-like growth factors) that act in turn on cancer cells to promote proliferation. The miRNA-30 family that is expressed at high levels in hormone-dependent and well-differentiated breast tumours has been described as a tumour suppressor in various solid tumours.[18] These miRNAs are also reported as osteomiRs that regulate osteoblast differentiation.[49] We have shown that miRNA-30s are downregulated in MDA-B02 cell line, a sub-population of the parental MDA-MB-231 breast cancer cells that specifically form bone metastasis in mice.[50] Restoring miRNA-30s in these cells decreased bone metastasis *in vivo* predominantly through the downregulation of genes associated with osteomimicry, such as integrinβ3, CTGF, cadherin-11, connexin 43 and RunX2. Osteomimicry regulation has been also reported for miRNA-218, an osteomiR that targets the WNT inhibitor SOST, DKK2 and SFRP2, thus enhancing WNT activity and abnormal expression of osteoblastic genes.[51] Upregulation of Ras-mediated signalling induces the aberrant activation of WNT activity in advanced prostate cancer. The WNT signalling-related gene, TCF7 is a critical factor of bone metastasis and a direct target of miR-34a. Ectopic miR-34a expression inhibits bone metastasis and reduces tumour burden in a Ras-dependent xenograft model by activating WNT and anti-apoptotic pathways.[52] Reintroduction of miR-203 in prostate tumour cells downregulated the expression of Zeb2, Bmi, Survivin and Runx2 that attenuates bone metastasis in mice.[53] Tumour-induced bone resorption stimulates the release of transforming growth factor-β, which in turn induces cancer cells to produce the pro-osteolytic factor IL-11. MiR-204, miR-211 and miR-379 are downregulated in a highly bone metastatic variant of the MDA-MB-231 cell line; these miRNAs were identified as potent inhibitors of transforming growth factor-β-induced IL-11 secretion.[54] MiR-34a is downregulated during osteoclast differentiation and has been identified as a critical suppressor of osteoclastogenesis, bone resorption and bone metastasis by directly targeting the pro-osteoclast factor Tgif2.[55] In lung cancer, Kuo *et al.*[56] have reported that PTHrP, a potent stimulator of osteoclastic bone resorption, is a direct target of miR-33a. MiR-33a levels are inversely correlated with PTHrP in lung cancer cell lines. Restoring miR-33a expression in lung cancer cells decreases the production of PTHrP and IL-8, another pro-osteoclastic factor. Thus, a low miR-33a expression contributes to cancer-mediated bone destruction and may predict a poor prognosis for lung cancer patients.[56] The validity of measuring serum miRNAs in a murine model of human lung cancer bone metastasis has been assessed by comparing miRNA serum levels with those of standard biochemical markers of bone turnover such as PINP, BGP (osteocalcin) and CTX.[57] In this model of lung cancer, PINP exhibited a strong correlation with osteolytic lesions and tumour burden at early and late stages of bone colonisation. In contrast, BGP and CTX demonstrated a strong correlation only at late stages. Interestingly, serum level of miR-326 was strongly associated with tumour burden and PINP in vehicle-treated animals, whereas no association was found in animals treated with an inhibitor of bone resorption. These results suggest that miR-326 could serve as a biochemical marker for monitoring bone metastatic progression in advanced lung cancer.[57]

Table 1 MiRNAs as clinical biomarkers of bone disease and potential therapeutic targets in breast, prostate and lung cancers

Role[a]	*miRNA*	*Activity*	*Targets*	*Predicted bone metastasis biomarkers in vivo therapeutic assays*	*Ref*
Breast cancer					
Tumour suppressors	miR-335	Invasion, metastatic seeding (Down)	Sox4, Tnc	Low level in tumour was associated with poor patient survival.	34
	miR-126	Proliferation, angiogenesis (Down)	IGFBP2, PITPNC1, MERTK	Silenced in breast cancer.	35
	miR-31	Invasion, metastatic seeding (Down)	RDX, RhoA, ITGA5	Levels in primary tumour was inversely correlated with metastasis occurrence.	45
	miR-30s	Invasion, osteomimicry (Down)	ITGβ3, CTGF, CDH11, RunX2	High levels are associated to ER+/PG+ in tumours.	18, 50
	miR-141, miR-219, miR-190	Osteoclastogenesis, tumour-induced bone resorption (Down)	Markers of osteoclast differentiation and activity	Intravenous delivery in mice inhibited osteoclast activity and reduced tumour burden and bone osteolysis.	22
	miR-34a	Osteoclast differentiation, bone metastasis (Down)	Tgif2	Knockdown of miR-34a in transgenic mice resulted in increased osteoclastogenesis.	55
Oncogenes	miR-103/107	EMT, migration, metastatic seeding miR biosynthesis (Down)	Dicer	High level in tumour was associated with poor patient survival.	21
	miR-10b	Invasion (Up)	HoxD10, RhoC	Increased levels were detected in metastatic tumours, with higher serum concentrations from patients with bone metastasis. Therapeutic silencing inhibited metastasis in a mouse mammary tumour model.	36, 44, 64
	miR-373/520c	Migration, invasion (Up)	CD44	Upregulation of miR-373 in metastasis was inversely correlated with CD44 expression.	40
	miR-17-92 cluster	Migration, metastatic seeding (Up)	TbR2	Anti-miR-17 inhibited metastasis to bone *in vivo* in a mouse model of human breast cancer.	41
Prostate cancer					
Tumour suppressors	miR-34a	Tumour-initiating and stem cell abilities (Down)	CD44	Tail vein delivery inhibited metastasis and extended animal survival.	30
	miR-143, miR-145	Migration, invasion, EMT (Down)	E-cadherin, fibronectin	Decreased in bone metastasis, expression negatively correlated to Gleason core and free PSA in primary tumour.	31, 32
	miR-203	Migration, invasion, osteomimicry (Down), MET (Up)	Survivin, Zeb2, Bmi1, RunX2, DlX5	Expression is decreased in bone metastasis and primary tumour, negatively correlated with advanced disease.	53
Oncogenes	miR-154[a], miR-379, miR-409-3p/5p	Tumour growth, EMT (Up)	Multiple signalling pathways	Elevated expression in bone metastatic tissues, correlated with progression-free survival of patients.	33
	miR-16	Proliferation (Down)	Cell cycle genes	Delivery of atelocollagen in mice tail vein decreased bone tumour burden.	71
Lung cancer					
Tumour suppressors	miR-192	Angiogenesis (Down)	IL-8, ICAM, CXCR4	*In vivo* infusion of exosome-like vesicles decreased bone lesions.	62
Oncogenes	miR-326	Decreased bone tumour and osteolytic lesions	unknown	Level associated with serum biochemical markers of bone turnover in a mouse model of bone metastasis.	57

Abbreviations: EMT, epithelial to mesenchymal transition; MET, mesenchymal-to-epithelial transition; PSA, prostate-specific antigen.
[a]OncomiRs are indicated by the ability to positively impact metastatic dissemination and bone metastasis, whereas tumour suppressors negatively impact metastatic dissemination and bone metastasis.

MiRNA-mediated regulation of stroma–tumour cell interactions in bone

There is strong evidence that miRNAs control the differentiation of osteoclasts and osteoblasts.[12] Recent studies have also demonstrated how these miRNAs may be transferred from the bone marrow stromal cells to nearby cancer cells through gap junction intracellular communication.[58] Co-culture of MDA-MB-231 or T47D breast cancer cells with bone marrow stromal cells resulted in cell cycle arrest in the G0 phase. Mir-127, -197, -222 and -223 that target CXCL12 are transported from the stromal cells to cancer cells leading to a decrease in proliferation that might explain the stroma-induced quiescence of breast cancer cells. Further, exosomes were isolated from bone marrow mesenchymal stem cellsand co-cultured with $CD44^+$-expressing MDA-MB-231 breast cancer cells, as a model of DTCs, to investigate interactions between cancer cells and the bone microenvironment.[59] In another study, the growth of CD44-expressing cells injected into the bone compartment was impaired in the presence of exosomes isolated from bone marrow mesenchymal stem cells. This was postulated to be due to elevated expression of exosome-derived miRNAs such as miR-23b, and was further substantiated when miR-23b overexpression partially recapitulated the tumour inhibitory effect *in vivo*. To further investigate cancer–stroma interactions, laser capture microdissection of breast cancer tissue showed an increase of miR-23b in adjacent bone marrow, thus suggesting proximity-based transfer of miRNA from the bone microenvironment to cancer cells in a clinical setting. Conversely, the stroma can promote metastatic tumour formation. In multiple myeloma, exosomes from bone marrow

mesenchymal stem cells promote tumour growth through the transfer of the oncogenic miR-15a in the bone compartment.[60] These studies provide evidence that the bone may be able to produce factors such as miRNAs that modulate the growth of DTCs. There are also reports that cancer cells can modify the microenvironment to become more favourable for metastatic tumour initiation. Conditioned media from highly metastatic cell lines are able to induce osteoclast differentiation.[22] Indeed, exposure of differentiating osteoclasts to tumour-conditioned media activates a specific miRNA signature in osteoclasts that is partly induced by the secretion of sICAM1 by bone-metastatic cells. This suggests that miRNAs from the tumour cells may also alter the bone microenvironment, disrupting bone turnover. Furthermore, secreted factors such as exosomal miRNAs may be released from tumour cells, which then pre-condition the bone microenvironment to become more favourable for tumour cell survival. Exosomal miR-135b from hypoxia-resistant multiple myeloma cell lines were able to enhance tube formation and induction of HIF-1 transcriptional activity through the inhibition of FIH-1 in human umbilical vein endothelial cells, implicating a role for miR-135b on the local microenvironment under hypoxic conditions.[61] In another study, pre-treatment of the intratibial compartment with exosome-bound miR-192 reduced bone lesions in immunocompromised mice, further confirming that secreted factors derived from miR-192-over-expressing cells promote osteolytic lesions and bone colonisation, presumably by targeting genes that act in tumour-induced angiogenesis *in vivo*.[62] The hypothesis that circulating miRNAs may pre-condition the metastatic niche to facilitate tumour growth is further strengthened by intravenous injection of exosomes from miR-192-overexpressing tumour cells, resulting in decreased bone lesion area and tumoural volume. Taken together, these studies illustrate how crosstalk between tumour cells and stromal cells can promote or inhibit tumour growth through the release of factors, such as miRNAs, which are able to act in a cell-autonomous manner.

MiRNAs as Clinical Biomarkers and Potential Therapeutic Targets in Bone Metastasis

We have reviewed how aberrant miRNA expression in primary tumours, bone marrow stroma and in secreted-circulating molecules of patients suffering from breast, prostate and lung cancer correlates with biological and clinical-pathological features (**Table 1**). Such observations have suggested that miRNAs could be used as diagnostic and prognostic biomarkers for patients with high risk of bone metastasis or relapse and for evaluation of treatment efficiency.[12,13] Evidence reporting that the profile of secreted miRNAs reflects their misexpression pattern both in tumour cells and bone stroma cells suggest the exciting possibility of using them as valuable index of the disease in regard of their stability and minimally invasive accessibility.[63] MiRNA expression *in vitro* and *in vivo* in mice preclinical models has been modulated by synthetic chemically miRNAs as both targets and tools (**Table 1**). Exogenous stabilised double-stranded mimics have been delivered in mice to augment miRNAs having tumour suppressor abilities. Recently, Ell *et al.*[22] have reported that the intravenous delivery of synthetic miRNAs in mice reduces osteolytic bone lesions by inhibiting osteoclast miRNAs. Strategy of inhibition of mature miRNAs by modified (2'-O-methyl/2'-O-methoxyethyl/locked nucleic acid) anti-sense oligonucleotides or by antagomirs (cholesterol-conjugated) has allowed the silencing of oncomirs both *in vitro* and *in vivo*. Administration of miR-10b antagomirs to mice bearing highly metastatic cells drastically reduces lung metastatic dissemination without any adverse effect in the animals.[64] Several other vector-based miRNAs delivering strategy have been developed to restore tumour suppressor or silence oncomiRNAs.[65] An elegant approach, the miRNA Sponge that inhibits miRNA activity for entire miRNA family by saturating them with target mRNAs has been developed by Ebert *et al.*[66] These plasmid vectors encode for mRNAs containing multiple tandem binding sites for endogenous mRNAs. Thus, during the cell miRNA processing, the Sponge interacts with the corresponding miRNA and prevents the association with the mRNA targets. Although miRNA-based therapeutic seems promising, their effective and safe delivery to tissue need further investigations. A number of clinical trials are underway in cancer to investigate the utility of miRNAs as disease markers or therapeutic targets. This includes miR-16, which has previously been discussed to be elevated in serum from breast cancer patients with bone metastasis.[22] Therapeutic targeting of miR-16 delivered in minicells (nanoparticles) has commenced Phase I trials in patients with malignant pleural mesothelioma and non-small cell lung cancer.[67,68] In addition to cancer biomarkers, clinical trials investigating miRNA expression levels have also commenced in other bone diseases and pathologies, such as postmenopausal osteoporosis[69] and monitoring bisphosphonate use during orthopaedic and oral maxillofacial surgery.[70,71]

Concluding Remarks

Bone metastasis is a multi-step process involving a series of molecular events that might already be genetically determined in primary tumours. The misexpression of miRNAs in tumour-initiating cells might up- or downregulate master genes that drive invasion, extravasation and finally bone seeding of cancer cells. Besides these regulatory mechanisms within tumour cells themselves, miRNAs interfere with tumour–stroma crosstalks within the bone. In this way, stroma–tumour interactions may be mediated by miRNA transfer through cell-to-cell interactions or exosomes to influence osteoclastogenesis and metastasis-related bone destruction.[72]

Identification of specific miRNA signatures in tumour cells that have bone-homing properties raises the possibility of developing miRNA-based biomarkers that could help identify patients with cancer at high risk of bone metastasis relapse. Further, the regulatory activity of miRNAs in the bone microenvironment implies that miRNAs could be promising therapeutic targets.

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References

1. Suva LJ, Washam C, Nicholas RW, Griffin RJ. Bone metastasis: mechanisms and therapeutic opportunities. *Nat Rev Endocrinol* 2011; **7**: 208–218.
2. Clezardin P, Teti A. Bone metastasis: pathogenesis and therapeutic implications. *Clin Exp Metastasis.* 2007; **24**: 599–608.
3. Coleman R, Gnant M, Morgan G, Clezardin P. Effects of bone-targeted agents on cancer progression and mortality. *J Natl Cancer Inst.* 2012; **104**: 1059–1067.
4. Clezardin P. Therapeutic targets for bone metastases in breast cancer. *Breast Cancer Res* 2011; **13**: 207.
5. Buijs JT, van der Pluijm G. Osteotropic cancers: from primary tumor to bone. *Cancer Lett.* 2009; **273**: 177–193.
6. Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. *Science* 2011; **331**: 1559–1564.
7. Nutter F, Holen I, Brown HK, Cross SS, Evans CA, Walker M *et al.* Different molecular profiles are associated with breast cancer cell homing compared with colonisation of bone: evidence using a novel bone-seeking cell line. *Endocr Relat Cancer.* 2014; **21**: 327–341.
8. Ell B, Kang Y. SnapShot: Bone Metastasis. *Cell* 2012; **151**: 690–690e1.
9. Smid M, Wang Y, Klijn JGM, Sieuwerts AM, Zhang Y, Atkins D *et al.* Genes associated with breast cancer metastatic to bone. *J Clin Oncol* 2006; **24**: 2261–2267.
10. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281–297.
11. Profumo V, Gandellini P. MicroRNAs: cobblestones on the road to cancer metastasis. *Crit Rev Oncog.* 2013; **18**: 341–355.
12. Ell B, Kang Y. MicroRNAs as regulators of bone homeostasis and bone metastasis. *BoneKEy Rep* 2014; **3**: 549.
13. Browne G, Taipaleenmäki H, Stein GS, Stein JL, Lian JB. MicroRNAs in the control of metastatic bone disease. *Trends Endocrinol Metab* 2014; **18**: 320–327.
14. Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordón-Cardo C *et al.* A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell.* 2003; **3**: 537–549.
15. Zhang XH-F Jin X, Malladi S, Zou Y, Wen YH, Brogi E *et al.* Selection of bone metastasis seeds by mesenchymal signals in the primary tumor stroma. *Cell* 2013; **154**: 1060–1073.
16. Aguirre-Ghiso JA. Models, mechanisms and clinical evidence for cancer dormancy. *Nat Rev Cancer.* 2007; **7**: 834–846.
17. Waning DL, Mohammad KS, Guise TA. Cancer-associated osteoclast differentiation takes a good look in the miR(NA)ror. *Cancer Cell.* 2013; **24**: 407–409.
18. Iorio MV, Ferracin M, Liu C-G, Veronese A, Spizzo R, Sabbioni S *et al.* MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 2005; **65**: 7065–7070.
19. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet.* 2010; **11**: 597–610.
20. Valastyan S, Weinberg RA. Metastasis suppression: a role of the Dice(r). *Genome Biol.* 2010; **11**: 141.
21. Martello G, Rosato A, Ferrari F, Manfrin A, Cordenonsi M, Dupont S *et al.* A MicroRNA targeting dicer for metastasis control. *Cell* 2010; **141**: 1195–1207.
22. Ell B, Mercatali L, Ibrahim T, Campbell N, Schwarzenbach H, Pantel K *et al.* Tumor-induced osteoclast miRNA changes as regulators and biomarkers of osteolytic bone metastasis. *Cancer Cell.* 2013; **24**: 542–556.
23. Mani SA, Guo W, Liao M-J, Eaton EN, Ayyanan A, Zhou AY *et al.* The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; **133**: 704–715.
24. Alix-Panabières C, Pantel K. Challenges in circulating tumour cell research. *Nat Rev Cancer.* 2014; **14**: 623–631.
25. Kang Y, Pantel K. Tumor cell dissemination: emerging biological insights from animal models and cancer patients. *Cancer Cell.* 2013; **23**: 573–581.
26. Li F, Tiede B, Massagué J, Kang Y. Beyond tumorigenesis: cancer stem cells in metastasis. *Cell Res.* 2007; **17**: 3–14.
27. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G *et al.* The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol.* 2008; **10**: 593–601.
28. Brabletz S, Brabletz T. The ZEB/miR-200 feedback loop--a motor of cellular plasticity in development and cancer? *EMBO Rep* 2010; **11**: 670–677.
29. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C *et al.* let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell* 2007; **131**: 1109–1123.
30. Liu C, Kelnar K, Liu B, Chen X, Calhoun-Davis T, Li H *et al.* The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. *Nat Med* 2011; **17**: 211–215.
31. Peng X, Guo W, Liu T, Wang X, Tu X, Xiong D *et al.* Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. *PloS ONE* 2011; **6**: e20341.
32. Guo W, Ren D, Chen X, Tu X, Huang S, Wang M *et al.* HEF1 promotes epithelial mesenchymal transition and bone invasion in prostate cancer under the regulation of microRNA-145. *J Cell Biochem.* 2013; **114**: 1606–1615.
33. Gururajan M, Josson S, Chu C-Y, Lu C-L, Lu Y-T, Haga CL *et al.* miR-154* and miR-379 in the DLK1-DIO3 microRNA mega-cluster regulate epithelial to mesenchymal transition and bone metastasis of prostate cancer. *Clin Cancer Res* 2014; **20**: 6559–6569.
34. Tavazoie SF, Alarcón C, Oskarsson T, Padua D, Wang Q, Bos PD *et al.* Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 2008; **451**: 147–152.
35. Png KJ, Halberg N, Yoshida M, Tavazoie SF. A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. *Nature* 2012; **481**: 190–194.
36. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 2007; **449**: 682–688.
37. Oskarsson T, Batlle E, Massagué J. Metastatic stem cells: sources, niches, and vital pathways. *Cell Stem Cell.* 2014; **14**: 306–321.
38. Hiraga T, Ito S, Nakamura H. Cancer stem-like cell marker CD44 promotes bone metastases by enhancing tumorigenicity, cell motility, and hyaluronan production. *Cancer Res.* 2013; **73**: 4112–4122.
39. Clezardin P. Integrins in bone metastasis formation and potential therapeutic implications. *Curr Cancer Drug Targets.* 2009; **9**: 801–806.
40. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S *et al.* The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. *Nat Cell Biol.* 2008; **10**: 202–210.
41. Liu S, Goldstein RH, Scepansky EM, Rosenblatt M. Inhibition of rho-associated kinase signaling prevents breast cancer metastasis to human bone. *Cancer Res.* 2009; **69**: 8742–8751.
42. Dangi-Garimella S, Yun J, Eves EM, Newman M, Erkeland SJ, Hammond SM *et al.* Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7. *EMBO J.* 2009; **23**: 347–358.
43. Croset M, Goehrig D, Frackowiak A, Bonnelye E, Ansieau S, Puisieux A *et al.* TWIST1 expression in breast cancer cells facilitates bone metastasis formation. *J Bone Miner Res* 2014; **29**: 1886–1899.
44. Zhao F-L, Hu G-D, Wang X-F, Zhang X-H, Zhang Y-K, Yu Z-S. Serum overexpression of microRNA-10b in patients with bone metastatic primary breast cancer. *J Int Med Res.* 2012; **40**: 859–866.
45. Valastyan S, Chang A, Benaich N, Reinhardt F, Weinberg RA. Activation of miR-31 function in already-established metastases elicits metastatic regression. *Genes Dev.* 2011; **25**: 646–659.
46. Bellahcène A, Bachelier R, Detry C, Lidereau R, Clézardin P, Castronovo V. Transcriptome analysis reveals an osteoblast-like phenotype for human osteotropic breast cancer cells. *Breast Cancer Res Treat.* 2007; **101**: 135–148.
47. Weilbaecher KN, Guise TA, McCauley LK. Cancer to bone: a fatal attraction. *Nat Rev Cancer.* 2011; **11**: 411–425.
48. Clezardin P. [The role of RANK/RANKL/osteoprotegerin (OPG) triad in cancer-induced bone diseases: physiopathology and clinical implications]. *Bull Cancer (Paris)* 2011; **98**: 837–846.
49. Wu T, Zhou H, Hong Y, Li J, Jiang X, Huang H. miR-30 family members negatively regulate osteoblast differentiation. *J Biol Chem.* 2012; **287**: 7503–7511.
50. Croset M, Bachelier R, Allioli N, Song SS, Agami R, Clezardin P. Targeting breast cancer osteomimetic genes by miRNA-30s decreases the formation of metastatic osteolytic lesions in mice 2011; **98**: S29–S30.
51. Hassan MQ, Maeda Y, Taipaleenmaki H, Zhang W, Jafferji M, Gordon JAR *et al.* miR-218 directs a Wnt signaling circuit to promote differentiation of osteoblasts and osteomimicry of metastatic cancer cells. *J Biol Chem.* 2012; **287**: 42084–42092.
52. Chen W-Y, Liu S-Y, Chang Y-S, Yin JJ, Yeh H-L, Mouhieddine TH *et al.* MicroRNA-34a regulates WNT/TCF7 signaling and inhibits bone metastasis in Ras-activated prostate cancer. *Oncotarget* 2014; **6**: 441–457(Advance Publications).
53. Saini S, Majid S, Yamamura S, Tabatabai L, Suh SO, Shahryari V *et al.* Regulatory role of mir-203 in prostate cancer progression and metastasis. *Clin Cancer Res* 2011; **17**: 5287–5298.
54. Pollari S, Leivonen S-K, Perälä M, Fey V, Käkönen S-M, Kallioniemi O. Identification of microRNAs inhibiting TGF-β-induced IL-11 production in bone metastatic breast cancer cells. *PloS ONE* 2012; **7**: e37361.
55. Krzeszinski JY, Wei W, Huynh H, Jin Z, Wang X, Chang T-C *et al.* miR-34a blocks osteoporosis and bone metastasis by inhibiting osteoclastogenesis and Tgif2. *Nature* 2014; **512**: 431–435.
56. Kuo P-L, Liao S-H, Hung J-Y, Huang M-S, Hsu Y-L. MicroRNA-33a functions as a bone metastasis suppressor in lung cancer by targeting parathyroid hormone related protein. *Biochim Biophys Acta.* 2013; **1830**: 3756–3766.
57. Valencia K, Martín-Fernández M, Zandueta C, Ormazábal C, Martínez-Canarias S, Bandrés E *et al.* miR-326 associates with biochemical markers of bone turnover in lung cancer bone metastasis. *Bone* 2013; **52**: 532–539.
58. Lim PK, Bliss SA, Patel SA, Taborga M, Dave MA, Gregory LA *et al.* Gap junction-mediated import of microRNA from bone marrow stromal cells can elicit cell cycle quiescence in breast cancer cells. *Cancer Res.* 2011; **71**: 1550–1560.
59. Ono M, Kosaka N, Tominaga N, Yoshioka Y, Takeshita F, Takahashi RU *et al.* Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. *Sci Signal* 2014; **7**: ra63.
60. Roccaro AM, Sacco A, Maiso P, Azab AK, Tai Y-T, Reagan M *et al.* BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. *J Clin Invest.* 2013; **123**: 1542–1555.
61. Umezu T, Tadokoro H, Azuma K, Yoshizawa S, Ohyashiki K, Ohyashiki JH. Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. *Blood* 2014; **124**: 3748–3757.
62. Valencia K, Luis-Ravelo D, Bovy N, Antón I, Martínez-Canarias S, Zandueta C *et al.* miRNA cargo within exosome-like vesicle transfer influences metastatic bone colonization. *Mol Oncol* 2014; **8**: 689–703.
63. Nana-Sinkam SP, Croce CM. Non-coding RNAs in cancer initiation and progression and as novel biomarkers. *Mol Oncol* 2011; **5**: 483–491.
64. Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG *et al.* Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. *Nat Biotechnol.* 2010; **28**: 341–347.
65. Iorio MV1, Casalini P, Piovan C, Braccioli L, Tagliabue E. Breast cancer and microRNAs: therapeutic impact. *Breast* 2011; **Suppl 3**: S63–S70.
66. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods.* 2007; **4**: 721–726.

67. Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J *et al.* Restoring expression of miR-16: a novel approach to therapy for malignant pleural mesothelioma. *Ann Oncol.* 2013; **24**: 3128–3135.
68. MesomiR 1: A Phase I Study of TargomiRs as 2nd or 3rd Line Treatment for Patients With Recurrent MPM and NSCLC. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [6 March, 2015]. Available from https://clinicaltrials.gov/show/NCT02369198NLM Identifier: NCT02369198..
69. Study on the microRNA Expression Level in Postmenopausal Osteoporosis. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000-[6 March, 2015]. Available from http://clinicaltrials.gov/show/NCT02128009NLM Identifier: NCT02128009.
70. Biomarker Identification in Orthopaedic & Oral Maxillofacial Surgery Subjects to Identify Risks of Bisphosphonate Use. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [6 March, 2015]. Available from http://clinicaltrials.gov/show/NCT01875458NLM Identifier: NCT01875458..
71. Takeshita F, Patrawala L, Osaki M, Takahashi RU, Yamamoto Y, Kosaka N *et al.* Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. *Mol Ther.* 2010; **18**: 181–187.
72. Mundy GR. Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat Rev Cancer.* 2002; **2**: 584–593.