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RESEARCH PAPER

Bone metastasis: pathogenesis and therapeutic implications

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Abstract Advanced cancers are prone to metastasize. Visceral metastases are more likely to be fatal, while patients with only metastases to bone can survive up to 10 years or more. However, effective treatments for bone metastases are not yet available and bisphosphonates improve the quality of life with no life-prolonging benefits. Bone metastases are classified as osteolytic, osteosclerotic or mixed lesions according to the bone cell types more prominently involved. Either conditions induce high morbidity and dramatically increase the risk of pathological fractures. Several molecular mechanisms bring about cancer cells to metastasize to bone, and osteotropic cancer cells are believed to acquire bone cell-like properties which improve homing, adhesion, proliferation and survival in the bone microenvironment. The acquisition of a bone cell pseudo-phenotype, denominated osteomimicry, is likely to rely on expression of osteoblastic and osteoclastic genes, thus requiring a multigenic programme. Several microenvironmental factors improve the ability of cancer cells to develop at skeletal sites, and a reciprocal deleterious stimulation generates a vicious cycle between the tumour cells and the cells residing in the bone environment. The impact of the stem cell niche in the development of bone metastases and in the phenomenon of tumour dormancy, that allows tumour cells to remain quiescent for decades before establishing overt lesions, is at present only speculative. However, the osteoblast niche, known to maintain the haematopoietic stem cell population in a quiescent status, is likely to be involved in the development of bone metastases and this promising research field is rapidly expanding.

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Abbreviations

BMP	Bone morphogenetic protein
BSP	Bone sialoprotein
CDH11	Cadherin 11
Cox-2	Cycloxygenase 2
CXCL-12	Chemokine (C-X-C motif) ligand 12
CXCR4	Chemokine (C-X-C motif) receptor 4
Cx43	Connexin 43
DKK-1	Dickkopf
FGF	Fibroblast growth factor
MSX2	Homeo box homolog 2
OPG	Osteoprotegerin
PDGF	Platelet derived growth factor
PTHrP	Parathyroid hormone related peptide
RANK	Receptor activator of nuclear factor-κB
RANKL	Receptor activator of nuclear factor-κB ligand
Runx2	Runt-related transcription factor 2
SNO	Spindle-shaped *N*-cadherin positive osteoblast
SPARC	Secreted protein, acidic, cysteine-rich (osteonectin)
TGFβ	Transforming growth factor β
VEGF	Vascular endothelial growth factor
Wnt	Wingless-type protein-1

Introduction

Advanced cancers are prone to metastasize [1–3]. Although metastatic cells could theoretically intrude any organ, clinical experience demonstrates that they have preference for lung (20–54% incidence) [4], liver (30–70% incidence) [5], bone (20% incidence) [6, 7], brain (15–72% incidence) [8] and adrenal gland (10–50% incidence) [9]. However, the homing to bone is much higher for certain cancers, including breast, prostate, lung and thyroid carcinomas, for which the likelihood to develop bone metastases increases considerably and the incidence is as high as 70% [10].

Visceral metastases are more likely to be fatal, with a long-term survival falling from 90 to around 5% [11]. In contrast, patients with only metastases to bone can survive up to 10 years or more [12–16]. In some patients, bone metastases develop many years after the surgical removal of the primary tumour, suggesting that the osteotropic malignant cells may have a long period of quiescence before developing the secondary lesion [17, 18]. Nevertheless, patients with overt bone metastases present with severe symptoms, including intractable bone pain, nerve compression syndromes, hypercalcaemia and pathological fractures, which considerably reduce the quality of life [10].

Effective treatments for bone metastases are not yet available. Bisphosphonates have demonstrated clinical utility in the palliative treatment of patients with bone metastases. They decrease skeletal morbidity (bone pain, pathological fractures), leading to an improvement of the quality of life but, unfortunately, they do not provide a life-prolonging benefit to patients with advanced cancer [19, 20]. Therefore, development of new therapeutics is required and, to achieve this goal, profound insights into the molecular mechanisms underlying the formation of bone metastases should be provided. Here, we describe cellular features associated with bone metastases, analyse the main molecular determinants known to impact on bone metastasis formation, and discuss our perspective for future molecularly-targeted therapeutic approaches.

Classification and cellular features of bone metastases

In bone metastases, metastatic cells actually intrude the bone marrow cavity where they grow forming a secondary lesion [21]. The mineralised nature of the bone tissue would theoretically prevent growing metastatic cells from forming wide tumours. This circumstance is however circumvented by a tight relationship between metastatic cells and bone cells which leads to microenvironmental changes that promote the enlargement of the bony cavity, thus creating more space suitable for tumour growth [21].

Bone metastases are classified as osteolytic, osteosclerotic or mixed lesions [21, 22] (Fig. 1). Osteolytic metastases are typical of breast cancer. They are caused by tumour-derived factors (Table 1) that stimulate the activity of bone-resorbing cells, the osteoclasts, leading to enhanced bone destruction [21, 25]. Radiographically, osteolytic lesions appear as radiolucent areas, frequently located in the skull and proximal ends of the long bones. Histologically, tumour cells reside in the bone marrow, and are surrounded by a number of osteoclasts, actively degrading bone. The progression of osteolytic lesions ultimately leads to the complete destruction of the bone wall and tumour cells can then extrude the bone cavity infiltrating the surrounding tissues. These osteolytic areas frequently fracture even in the absence of traumas [21, 22].

Osteosclerotic metastases are more typical of prostate cancer and are caused by cancer-derived factors (Table 1) that stimulate the differentiation and activity of bone-forming cells, the osteoblasts, thus leading to increased bone formation [21, 22]. Radiographically, osteosclerotic lesions appear as dense areas, often located to the axial skeleton and, particularly, in vertebral bodies and pelvis. Histologically, tumour cells residing in the bone marrow are surrounded by a high number of osteoblasts that form wide trabeculae of woven bone similar to that observed in primary ossification. Tumour-associated woven bone has however a poorly organised microstructure, increasing again the risk of pathological fractures [21, 22].

Bone resorption and bone formation are almost always coupled [26]. This coupling is a dynamic process, which is altered in cancer, thereby leading to skeletal lesions that are predominantly osteolytic or osteoblastic. However, in many instances, bone metastases may consist of mixed lesions [21]. Indeed, it is believed that a bone metastasis may evolve from an osteoblastic to an osteolytic pattern through a continuous process of which we only have a static representation at the time of the radiographical or histological assessment.

Bone metastasis formation consists of a series of inter-related steps that begins with the tropism of cancer cells to the bone through specific migratory and invasive processes, then follows with the growth of cancer cells in the bone marrow which requires that these cells acquire “bone-like” or osteomimetic properties, and ends with bi-directional interactions between cancer cells, osteoclasts and osteoblasts which determine whether the subsequent bone metastasis is osteolytic or osteoblastic. Molecular mechanisms involved in each of these steps are gradually being unravelled, and are potential therapeutic targets for the prevention and treatment of bone metastases. Further complexities are introduced by the fact that the bone microenvironment does not only include the cellular architecture of the bone tissue but also bone marrow-derived haematopoietic progenitors and cancer stem cells that altogether constitute a niche supporting the development of metastases.

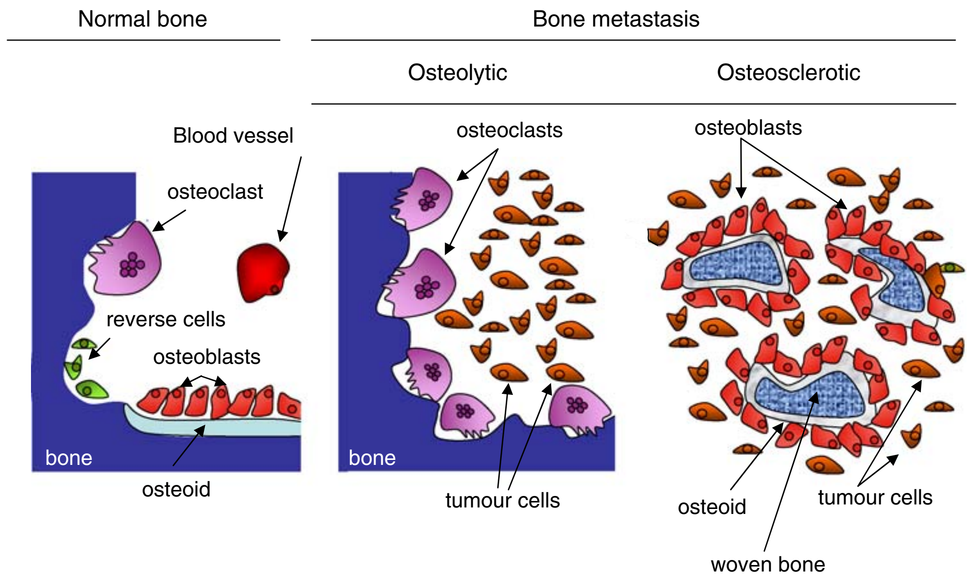

Fig. 1 *Classification of bone metastases*. *Left panel*: cartoon depicting a normal bone, in which osteoclasts and osteoblasts function in a concerted manner. Osteoclasts remove the old bone matrix, which, after a poorly defined reverse phase, populated by mononuclear cells (reverse cells) participating to the coupling between osteoclast and osteoblast activity, is replaced by new osteoid released by active osteoblasts, which eventually mineralises. *Middel panel*: in osteolytic bone metastases, an incredibly high number of osteoclasts are formed which resorb the mineralized matrix destroying the tissue. *Right panel*: in osteosclerotic bone metastases, numerous osteoblasts appear forming new trabeculae that occlude the bone marrow. This bone matrix has the histological and biochemical features of woven bone and is inordinately deposited in the medullary cavity. Mixed bone metastases (not shown) have both increased osteoclasts and osteoblats in close vicinity

Bone tropism

Different molecular mechanisms are responsible for the propensity of cancer cells to metastasize to bone. The chemokine receptor CXCR4 controls the metastatic destination of breast cancer cells in certain organs (lung, liver and bone marrow) where its ligand, the chemokine CXCL-12, is produced in high quantity [27]. Consistent with this, the blockade of CXCR4 using antibodies or a synthetic peptidic antagonist reduces the formation of experimental lung and bone metastases caused by CXCR4-expressing breast or prostate cancer cells [28, 29]. However, the inhibition of chemokine receptors in vivo only partially blocks metastasis formation, suggesting that additional factors are involved in the bone tropism of cancer cells. Indeed, bone-derived cytokine RANKL triggers the migration of RANK-expressing cancer cells in vitro, and osteoprotegerin (OPG), a natural inhibitor of RANK–RANKL interaction, blocks the bone tropism of these cancer cells in vivo [30]. There is also a growing body of evidence from preclinical research showing that integrins mediate metastasis to specific organs. For instance, we have recently shown that $\alpha v\beta 3$ integrin overexpression in breast cancer cells enhances bone metastasis incidence in animals, and that a nonpeptide $\alpha v\beta 3$ integrin antagonist causes a profound and specific inhibition of bone colonisation by $\alpha v\beta 3$-expressing cancer cells in vivo [31]. In a similar vein, bone colonisation by prostate cancer cells has been reported to be mediated by $\alpha 2\beta 1$ integrin [32]. Tumour $\alpha v\beta 3$ and $\alpha 2\beta 1$ integrins mediate the attachment of cancer cells to extracellular matrix proteins (BSP and type-I collagen, respectively). It is therefore possible that these integrins act in concert with CXCR4 and RANKL to promote the bone colonisation by cancer cells.

Another important determinant for bone tropism, also linked to integrin functions, is the proto-oncogene c-Src, a non-receptor tyrosine kinase, homologous to the viral oncogene v-Src [33]. It plays a role in cell growth, cytoskeletal remodelling, adhesion and motility [34]. Although ubiquitously expressed, c-Src deficiency appears to affect only the skeleton with no apparent effects on other organs [35]. In many tumours, c-Src is upregulated or hyperactivated thus affecting cancer cell properties linked to proliferation, motility and responses to growth factors [36]. Interestingly, comparing the transcriptomes of human breast cancer bone metastases versus visceral metastases, we observed that a subset of up-regulated genes are under the control of c-Src (MetaBre unpublished observations). In a similar vein, a clone of the parental MDA-MB-231 breast cancer cell line, which shows increased capacity of bone metastasis, also exhibits elevated c-Src protein and tyrosine phosphorylation of c-Src [37]. In addition, reduced

Table 1 Factors implicated in osteolytic and osteosclerotic metastases [23, 24]

Factor	Role in osteolytic metastasis	Role in osteoblastic metastasis
RANKL	Stimulates osteoclast formation, activity and survival	
OPG		Inhibits osteoclast formation and activity
M-CSF, MG-CSF	Stimulate monocytic lineage, osteoclast formation and survival	
PTHrP	Stimulates RANKL and inhibits OPG expression, enhancing osteoclast formation	
IL-1	Stimulates osteoclast formation, activation and survival	
IL-6	Stimulated osteoclast formation, activation and survival. Enhances IL-1 and IL-6 expression	
IL-8	Stimulates osteoclast formation	
IL-11	Stimulates osteoclast formation	
TNFα	Stimulates osteoclast formation	
Prostaglandins	Stimulates osteoclast formation	
CTGF	Induces expression of TGFβ, stimulates angiogenesis and bone resorption	
CXCL-12	Stimulates angiogenesis and tumour cell migration	
COX2	Induces prostaglandin E2, IL-8 and IL-11	
Osteopontin	Promotes osteoclast adhesion	
VEGF	Stimulates angiogenesis and osteoclast formation	Stimulates angiogenesis and osteoblast activity
Metallo-proteinases	Contribute to bone resorption	
Urokinase		Stimulates osteoblast proliferation
TFGβ	Complex role, ending up with increase of osteoclast formation. Promotes epithelial–mesenchymal transition	Promotes epithelial–mesenchymal transition. Recruits and stimulates osteoblasts
PDGF	Promotes epithelial–mesenchymal transition	Promotes epithelial–mesenchymal transition, angiogenesis and osteoblast activity
BMPs	Promote epithelial–mesenchymal transition	Promote epithelial–mesenchymal transition, osteoblast formation and activity
IGFs		Stimulate osteoblast activity
FGFs		Stimulate osteoblast activity
PSA		Stimulates TGFβ
Wnt		Stimulates osteoblasts, inhibits osteoclasts
DKK-1	Inhibits osteoblasts	
Noggin	Inhibits osteoblasts	
Endothelin 1		Stimulates osteoblasts, inhibits osteoclasts and potentiates the effect of growth factors

c-Src activity in breast cancer cells decreases their malignant phenotype and osteotropism in experimental metastases [37, 38], a circumstance that leads to the administration of c-Src inhibitor or biologic agent, which successfully slowed down the metastatic process [38–40].

Osteomimicry

The definition of osteomimicry is the acquisition by tumour cells of bone cell-like properties, which improve homing, adhesion, proliferation and survival in the bone microenvironment [41, 42] (Fig. 2). This is due to the ability of osteotropic malignant cells to express transcription factors (Runx2, MSX2) [43, 44] that are master regulators of osteoblast differentiation and strong inducers of the expression of bone proteins. For instance, bone matrix proteins, including osteopontin [45], osteocalcin [46], osteonectin [47] and bone sialoprotein II [48], are frequently highly expressed in breast and prostate cancers, which represent tumours with the highest propensity to colonise bone. In addition, these proteins are also highly expressed in human breast cancer cell lines that form experimental bone metastases when injected in immunocompromised mice. Minn et al. [49] have demonstrated that single cell populations obtained from the MDA-MB231 human breast cancer cell line, selected for their high osteotropism, express a unique set of genes, among which transcripts typical of the osteoblast phenotype are prominent. In addition, the ability of these single cell populations

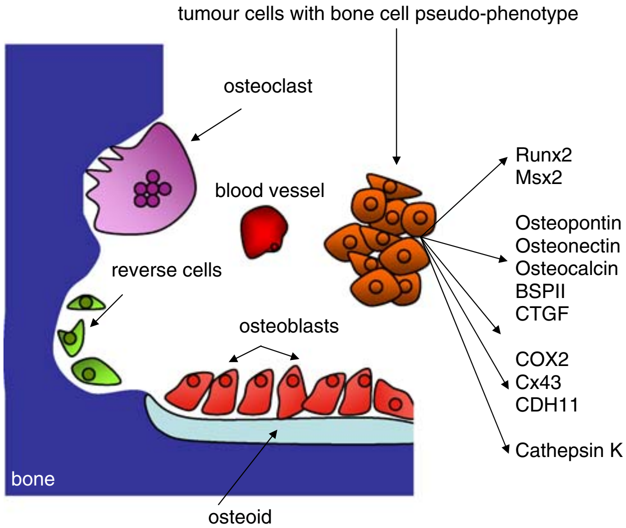

Fig. 2 *Multigenic programme of osteomimicry*. Osteotropic tumour cells acquire the ability to grow in the bone microenvironment because of their bone cell pseudo-phenotype due to the high expression of transcription factors (Runx2, MSX2), extracellular matrix proteins [osteopontin, osteonectin, osteocalcin, bone sialoprotein II (BSP II)], proteases (cathepsin K), and other bone-related factors (COX2, Cx43, CDH11) that, under physiological conditions, regulate osteoblast differentiation and osteoclast activity

to induce bone metastases is greatly enhanced when an osteomimetic gene, the osteopontin, is co-expressed along with either one of the genes of the osteotropic signature. Conversely, the blockade of Runx2 transcription factor in MDA-MB-231 cells, an inducer of osteopontin expression, inhibits breast cancer bone metastasis formation [50].

A global transcriptome analysis of another osteotropic MDA-MD231 cell variant, BO2, harvested from an experimental bone metastasis after injection of the parental cells in nude mice, has confirmed that several genes among those mostly up- or down-regulated relative to the parental cell line, correspond to genes whose expression is associated with the osteoblast differentiation process [51]. Most interestingly, the proteins encoded by a set of genes, including *CDH11*, *COX-2*, *CTGF*, *Cx43* and *SPARC*, which are overexpressed by BO2 cells and are known to be up-regulated during osteoblast differentiation, are also selectively overexpressed in human breast cancer bone metastases relative to the primary tumour and visceral (liver) metastases. Likewise, proteins encoded by genes underexpressed in BO2 cells and downregulated during osteoblast differentiation, are also selectively underexpressed by human breast cancer bone metastases relative to the primary tumours and visceral (liver) metastases [51].

In addition, a global gene profiling analysis performed on human metastatic tissues from breast carcinomas has established that there is a unique set of genes overexpressed in bone metastases compared to any other type of visceral metastases (liver, lung and brain) examined so far (MetaBre unpublished results). Among these genes, many have an osteomimetic significance and, for instance, the already known osteomimetic bone sialoprotein II is >100-fold overexpressed in bone versus visceral metastases (MetaBre unpublished results). Collectively, these data point to the osteomimetic properties of malignant cells as a key event that favours the development of a secondary lesion in the bone/bone marrow microenvironment.

The question however remains as these osteomimetic properties refer only to the osteoblast phenotype or if they can be extended to the osteoclast phenotype as well. For instance, others and we have shown that cathepsin K, a typical and highly specific osteoclast gene, is overexpressed in human breast cancer cells that metastasize to bone [52, 53]. Therefore, it is conceivable that a multigenic mimicry programme is indispensable for a tumour cell to develop in the bone, and that this programme includes both osteoblastic and osteoclastic genes.

Microenvironmental factors

What makes a tumour cell with a multigenic osteomimicry programme capable of developing a secondary bone lesion is probably associated with the favourable microenvironment [54]. Bone tissue is subjected to a continuous bone remodelling cycle in which osteoclasts resorb the old and damaged bone, and osteoblasts replace this bone with newly formed matrix [55–57]. These cycles are repeated life-long, therefore it is believed that the entire skeletal matrix is replaced several times during life. Many factors regulate bone remodelling, including systemic hormones and local factors, among which interleukins, cytokines, colony-stimulating factors, eicosanoids, the RANKL/OPG axis and PTHrP, play relevant roles [55–58]. In addition, many growth factors are synthesised by the osteoblasts and embedded into the bone matrix, mostly as inactive peptides, during the bone formation phase [55–57]. These factors, which include Transforming growth factor β (TFGβ), Platelet derived growth factor (PDGF), Bone morphogenetic proteins (BMPs) among others, are then released from the matrix during bone resorption and activated both by the low pH created by the osteoclasts to remove the bone mineral, and by a set of proteases present in the microenvironment [59]. Therefore, during bone resorption, the bone/bone marrow microenvironment is enriched by a plethora of agents regulating many cellular activities. In addition, it has to be noted that the bone and the bone marrow are tightly linked, and that bone cells and haematopoietic cells are reciprocally regulated and interconnected in their function [60, 61].

In 1889, Stephan Paget [62] had proposed that environmental factors provide a fertile ground (the soil) in which tumour cells (the seed) can grow. This "Seed and Soil" theory has been largely demonstrated by many studies over the century and is particularly true for osteotropic cancers [63]. Indeed, in bone, years ago the groups of Yoneda et al. [21], have recognized that a vicious cycle is established during the formation of bone metastases, consisting in the perturbation of the microenvironment initiated by the tumour cells that produce many factors stimulating the osteoclasts, with the end point of an increased bone resorption (Fig. 3). In addition, cancer cells secrete bone morphogenetic and Wnt protein antagonists (noggin, DKK-1) that inhibit osteoblast activity which, in turn, enhance the osteolytic pattern of bone metastases [32, 64]. Conversely, cancer cells (especially in the prostate) may release endothelin-1, which stimulates bone formation and inhibits bone resorption, leading to the formation of osteosclerotic lesions [65, 66]. Additional factors like Fibroblast growth factor (FGFs), PDGF and BMPs may be involved as well in the osteoblastic pattern of bone metastases [22]. As stated above, upon bone resorption, osteoclasts then release tumour-seeking factors from the matrix, which in turn stimulate proliferation, survival and migration of the tumour cells. In addition, many bone matrix-derived factors, including TGFβ, PDGF and BMPs, have the ability to induce the epithelial-mesenchyme transition of cancer cells, a key event that greatly enhances their malignant phenotype [67]. Finally, tumour cells have the ability to induce angiogenesis through the secretion of Vascular endothelial growth factor (VEGF) and FGFs [25]. It is therefore clear that the osteomimetic properties of cancer cells and their adaptation to survive in such an enriched environment, are key determinants for the development of bone lesions.

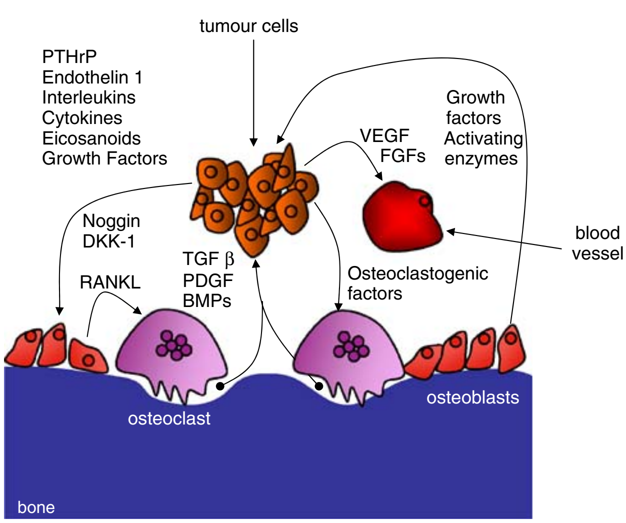

Fig. 3 *The vicious cycle*. The cartoon illustrates the many factors that reciprocally stimulate on one hand osteoclast, osteoblast and vascular cells activity, and on the other hand tumour cell growth and survival in the bone microenvironment

The impact of the stem cell niche

It is known that a well-defined stem cell hierarchy is responsible for normal tissue regeneration, which ends up with the repair of the tissues and replacement of worn-out cells in the organs [68]. Stem cells are also implicated in the development of cancer [69, 70]. Genome and population evolution in tumours is quite complex and requires multistep events. Random genetic and epigenetic changes are likely to occur in normal tissues by continuous proliferation, environmental stress, physiological changes and others. This is believed to lead to development of genetic heterogeneity, with mutations that in most cases remain neutral, and cells eventually die or persist without undergoing further transformation. However, some cells may harbour non-neutral mutations that stratify them into stem/progenitor clones or differentiated clones. Further mutations, perturbing the balance of self-renewal over quiescence in the former, or inducing loss of cell cycle control in the latter, may lead to a tissue that contains pre-tumour stem cells. These stem cells may generate a benign tumour or, if subjected to further genetic hits with mutations giving an advantage within the tissue niche, they give rise to a malignant tumour that retains deregulated stem cells. These cells have metastatic potential and, with further genetic hits and mutations that give an adaptive advantage at the secondary site, eventually form secondary tumours with niche dominance of specific clones [71]. Interestingly, it is believed that metastatic cells that achieve the bone marrow, are likely to remain dormant for many years before forming an overt bone metastases [72]. This seems to represent the backdrop why patients with certain cancers, including breast and prostate, develop bone metastases decades after the surgical removal of the primary tumour [73–76]. The molecular determinants influencing tumour stem cell quiescence in the bone marrow environment are yet to be elucidated. Understanding these mechanisms may help devising strategies to cure the disease or at least to induce persistent remission.

It is interesting to note that osteoblasts play a fundamental role in the quiescence of the long-term haematopoietic stem cell, that through cell–cell, cell–matrix and paracrine interactions with a subset of Spindle-shaped, *N*-cadherin positive osteoblasts (SNO) are kept associated to the endosteal bone surface and prevented to proliferate, until microenvironmental changes promote their detachment from SNO and the progression toward the myeloid and lymphoid lineages [77, 78]. SNO cells are thus suspected to provide a niche for haematopoietic stem cells, which, in combination with specific environmental factors, paracrine/autocrine signals and cellular determinants, provide inhibitory stimuli maintaining the long-term haematopoietic stem cell in a quiescent status [77, 78]. In contrast, the so-called vascular niche, represented by yet to be defined cells residing in the bone marrow sinusoidal system, appears to be involved in the rescue of intense proliferation and transition toward an active status [78]. It is thus tempting to hypothesise that a tumour stem cell intruding the bone marrow could be recruited by the SNO or SNO-like cells and kept quiescent, until environmental changes may restore their ability to enter the cell cycle (Fig. 4). It has to be noted that among the factors released by osteoclasts during bone resorption, there are molecules typically involved in the epithelial-mesenchyme transition (TFGβ, PDGF, BMPs). Therefore, osteoclast activation may play a critical role in the awakening of dormant tumour cells. Many environmental changes could elicit osteoclast activation, among which inflammation is most likely an important condition, which is known to increase bone resorption and reduce bone formation [79–81]. Should this circumstance be confirmed, it is imaginable that administration of anti-inflammatory drugs could represent a valid devise to keep tumour stem cell quiescent and induce persistent remission of the bone metastatic disease. In keeping with this hypothesis, COX2 inhibitors are effective for the treatment of experimental bone metastases [82], therefore we believe plausible that one mechanism of action could be prevention of stem cell activation.

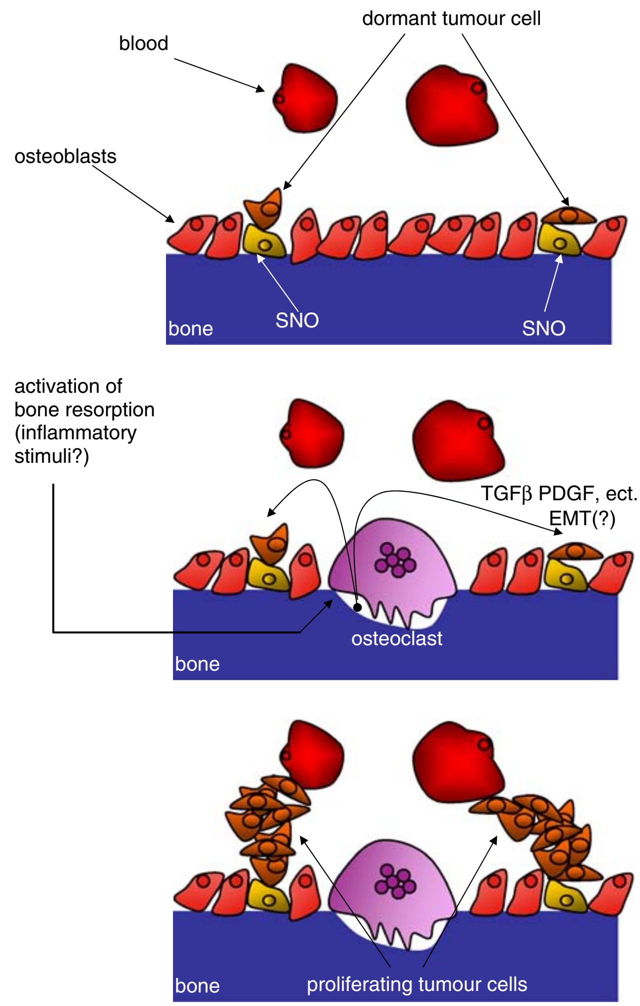

Fig. 4 *The role of the osteoblast niche in the control of tumour cell growth. Upper panel*: among the many osteoblasts lining the bone surface, a few are Spindle-shaped *N*-cadherin positive osteoblasts (SNO) implicated in the maintenance of haematopoietic stem cell quiescence (osteoblast niche). In this circumstance, scattered tumour cells reaching the bone marrow could be sequestered by the SNO and kept dormant. *Middle panel*: in the event that environmental conditions change and induce osteoclast formation and bone resorption, factors involved in the epithelial-mesenchyme transition may be released from bone matrix (or directly by the osteoclasts) and activate the quiescent tumour cells. RANKL and inflammatory cytokines are known to be potent osteoclast-inducing factors and could play a role in this context. *Lower panel*: activated tumour cells are then induced to proliferate progressing into an overt metastases. Whether or not the so-called vascular niche may play a role in tumour cell activation is presently unknown, but would match with the knowledge that the vascular niche is involved in activation of the haematopoietic stem cells and that tumour cells strongly depend on neoangiogenesis for their survival

Therapeutic implications and future perspectives

As mentioned above, there are many new exciting pathways that can be harmed to inhibit the development of bone metastases and more will be identified in the near future due to the tremendous effort in the field. New conventional drugs as well as innovative therapeutics are expected to provide effective tools to combat the disease (Table 2), cooperating with bisphosphonates or replacing them in new treatment protocols.

Besides anti-resorptive (bisphosphonates, cathepsin K inhibitor), anti-COX2, anti-CXCL-12, anti-integrin $\alpha V\beta 3$, anti-c-Src tyrosine kinase and anti-Runx2 experimental treatments already described above, it is worth mentioning here a few more therapies which have potential for future applications. For instance, denosumab is a fully human monoclonal antibody to RANKL that has high affinity and specificity for RANKL. Its mechanism of action is similar to OPG, which prevents RANKL from binding RANK, thus inhibiting osteoclast formation and reducing the incidence of osteolytic metastases [83]. It is a human IgG2 molecule with a long circulatory residence time, resulting in rapid and sustained decrease of bone resorption. After positive trials in which it was proven to reduce bone loss in postmenopausal osteoporosis, denosumab is now in phase II clinical studies for breast cancer and multiple myeloma bone metastases. It shows efficacy similar to that of

Table 2 Therapeutic agents[a]

Agents	Application	Role
Anti-resorptive	Clinical	Inhibit osteoclast formation and/or bone resorption. Active in osteolytic metastases
Anti-TGFβ	Preclinical	Inhibit TGFβ family members or their receptors. Active in osteolytic metastases in which they block the vicious cycle. Active in osteoblastic metastases in which they inhibit osteoblast recruitment and differentiation
Anti-inflammatory	Chemoprevention study	Reduce tumour cell activity, reduces bone resorption. Active in osteolytic metastases
Anti-angiogenesis	Clinical	Blocks development of new vessels. Potentially active in any type of metastases
Anti-CXCL-12	Preclinical	Blocks metastatic destination of cancer cells
Anti-$\alpha V\beta 3$ integrin	Clinical Phase I	Blocks bone colonization by cancer cells. Blocks angiogenesis
Anti-c-Src tyrosine kinase	Preclinical	Reduced proliferation, motility and responses to growth factors in cancer cells. Blocks bone resorption
Anti-Runx2	Preclinical	Blocks formation of bone metastasis

[a] References are in the text

bisphosphonates but with a better compliance and ease of treatment [84, 85].

Promising expectation is also provided by preliminary studies showing the efficacy of inhibitors of the TGFβ superfamily, which include natural inhibitors, soluble forms of the receptors, blocking antibodies and small chemical inhibitors directed towards the TGFβ family itself or their receptors [86, 87]. These inhibitors are being tested in a number of diseases whose pathogenesis is associated with misregulation of TGFβ family members, including cancer, muscular dystrophy, obesity and bone diseases, among which bone metastases appear good targets due to the prominent role of TGFβ in the development of both osteolytic and osteoblastic lesions.

Promise also emerges by the use of anti-angiogenetic agents. These have the advantage not to be restricted to specific tumour histotypes or sites of secondary lesions. They target endothelial cells, which are easy to reach by systemic treatment, and have limited side effects because physiologic angiogenesis occurs in adult only in certain circumstances such as the ovarian/uterin cycle and wound healing. It is, therefore, a selective therapy and the targeted endothelial cells are genetically stable, therefore they are unlikely to develop drug resistance. Two groups of compounds have been approved as antiangiogenic monotherapy for solid tumours: small-molecule kinase inhibitors, and humanized anti-VEGF monoclonal antibody [88]. Although they are not being specifically tested for bone metastases in clinical trials, PTK787 (a VEGF receptor tyrosine kinase inhibitor) decreases the formation of osteoblastic lesions in animals bearing C4-2B prostate tumours [89], suggesting these anti-angiogenic drugs have the potential to inhibit tumour spreading to bone similar to their action in other organs. Also interesting is the possibility of anti-angiogenic therapy preventing tumour stem cell activation, which is currently being tested in experimental models of xenograft tumours [90].

Finally, endothelin receptor antagonists are in phase II and III clinical trials for a wide range of solid tumours [91], including prostate cancer. Endothelin 1 is one of the most relevant inducer of prostate cancer osteosclerotic metastases, stimulating osteoblasts and potentiating the effect of growth factors [23]. It is thus possible that current clinical trials may unravel the potential for endothelin receptor antagonists to combat prostate cancer bone metastases.

Concluding remarks

In conclusion, bone metastases are likely to rely on: (i) the ability of cancer cells to exhibit specific receptor–ligand interactions that direct their homing to bone, (ii) the osteomimicry which, by exploiting a bone pseudo-phenotype, leads to tumour development in the bone/bone marrow tissue; (iii) the microenvironmental factors which establish a self-perpetuating vicious cycle and (iv) the stem cell niche which could maintain tumour stem cells dormant until permissive conditions arouse them. Future developments are expected to improve our knowledge on molecular determinants that are critical for bone metastasis formation and develop new therapeutics to combat cancer-induced bone diseases.

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References

1. Gupta GP, Massague J (2006) Cancer metastasis: building a framework. Cell 127:679–695
2. Steeg PS (2006) Tumor metastasis: mechanistic insights and clinical challenges. Nat Med 12:895–904

3. Eccles SA, Welch DR (2007) Metastasis: recent discoveries and novel treatment strategies. Lancet 369:1742–1757
4. Hassan I (2006) Lung, metastases. In: Shoffer K, Coombs BD, Webb R, Krasny R, White CR, (eds) e-Medicine specialties—radiology—chest (http://www.emedicine.com/radio/topic404.htm). Cited October 25, 2006
5. Khan AN, Macdonald S (2007) Liver, metastasis. In: Amin Z, Coombs BD, Schmiedl UP, Krasny, RM, Karani J (eds) e-Medicine specialties—radiology—liver (http://www.emedicine.com/radio/topic394.htm). Cited January 24, 2007
6. James JJ, Evans AJ, Pinder SE et al (2003) Bone metastases from breast carcinoma: histopathological–radiological correlations and prognostic features. Br J Cancer 89:660–665
7. Wilfred CG, Muttarak M (2007) Bone metastases. In: Abdel-Dayem HM, Coombs BD, Peh WCG, Krasny RM, Chew FS (eds) e-Medicine specialties—radiology—musculoskeletal (http://www.emedicine.com/radio/topic88.htm). Cited February 16, 2007
8. Khosla A (2007) Brain, metastases. In: Creasy JL, Coombs BD, DeLaPaz RL, Krasny RM, Smirniotopoulos JG (eds) e-Medicine specialties—radiology—brain/spine (http://www.emedicine.com/radio/topic101.htm). Cited January 24, 2007
9. Wansaicheong G, Goh J (2205) Adrenal metastases. In: Krinsky G Coombs BD, Friedman AC, Krasny RM, Lin EC (eds) e-Medicine specialties—radiology—genitourinary (http://www.emedicine.com/radio/topic17.htm). Cited January 3, 2005
10. Coleman RE (2006) Clinical features of metastatic bone disease and risk of skeletal morbidity. Clin Cancer Res 12:6243s–6249s
11. Greenberg PA, Hortobagyi GN, Smith TL et al (1996) Long-term follow-up of patients with complete remission following combination chemotherapy for metastatic breast cancer. J Clin Oncol 14:2197–2205
12. Fan K, Peng CF (1983) Predicting the probability of bone metastasis through histological grading of prostate carcinoma: a retrospective correlative analysis of 81 autopsy cases with antemortem transurethral resection specimen. J Urol 130:708–711
13. Coleman RE, Smith P, Rubens RD (1998) Clinical course and prognostic factors following bone recurrence from breast cancer. Br J Cancer 77:336–340
14. Koenders PG, Beex LV, Kloppenborg PW et al (1992) Human breast cancer: survival from first metastasis. Breast Cancer Study Group. Breast Cancer Res Treat 21:173–180
15. Solomayer EF, Diel IJ, Meyberg GC et al (2000) Metastatic breast cancer: clinical course, prognosis and therapy related to the first site of metastasis. Breast Cancer Res Treat 59:271–278
16. Cook RJ, Major P (2006) Multistate analysis of skeletal events in patients with bone metastases. Clin Cancer Res 12:6264s–6269s
17. Callaway MP, Briggs JC (1989) The incidence of late recurrence (greater than 10 years); an analysis of 536 consecutive cases of cutaneous melanoma. Br J Plast Surg 4246–4249
18. Slade MJ, Coombes RC (2007) The clinical significance of disseminated tumor cells in breast cancer. Nat Clin Pract Oncol 4:30–41
19. Dunstan CR, Felsenberg D, Seibel MJ (2007) Therapy insight: the risks and benefits of bisphosphonates for the treatment of tumor-induced bone disease. Nat Clin Pract Oncol 4:42–55
20. Khosa AD, Nayyar MS, Beirne JC (2007) Osteochemonecrosis of jaws and bisphosphonates. Ir Med J 100:410–411
21. Roodman GD (2004) Mechanism of bone metastases. N Eng J Med 350:1655–1664
22. Guise TA, Mohammad KS, Clines G et al (2006) Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin Cancer Res 12:6213s–6216s
23. Virk MS, Lieberman JR (2007) Tumor metastasis to bone. Arthritis Res Ther 9:S5
24. Rose AAN, Siegel PM (2006) Breast cancer-derived factors facilitate osteolytic bone metastasis. Bull Cancer 93:931–943
25. Ye L, Kynaston HG, Jiang WG (2007) Bone metastasis in prostate cancer: molecular and cellular mechanisms. Int J Mol Med 20:103–111
26. Karsdal MA, Martin TJ, Bollerslev J et al (2007) Are nonresorbing osteoclasts sources of bone anabolic activity? J Bone Miner Res 22:487–494
27. Muller A, Homey B, Soto H et al (2001) Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50–56
28. Liang Z, Wu T, Lou H et al (2004) Inhibition of breast cancer metastasis by selective synthetic polypeptide against CXCR4. Cancer Res 64:4302–4308
29. Sun YX, Schneider A, Jung Y et al (2005) Skeletal localization and neutralization of the SDF-1(CXCL12)/CXCR4 axis blocks prostate cancer metastasis and growth in osseous sites in vivo. J Bone Miner Res 20:318–329
30. Jones DH, Nakashima T, Sanchez OH et al (2006) Regulation of cancer cell migration and bone metastasis by RANKL. Nature 440:692–696
31. Zhao Y, Bachelier R, Treilleux I et al (2007) Tumor $\alpha v\beta 3$ integrin is a therapeutic target for breast cancer bone metastases. Cancer Res 67:5821–5830
32. Hall CL, Dai J, van Golen KL et al (2006) Type I collagen receptor ($\alpha 2\beta 1$) signaling promotes the growth of human prostate cancer cells within the bone. Cancer Res 66:8648–8654
33. Rucci N, Šuša M, Teti A (2007) Inhibition of protein kinase c-Src as a therapeutic approach for cancer and bone metastases. Anti-Cancer Agents in Med Chem (in press)
34. Homsi J, Cubitt C, Daud A (2007) The Src signaling pathway: a potential target in melanoma and other malignancies. Expert Opin Ther Targets 11:91–100
35. Soriano P, Montgomery C, Geske R et al (1991) Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 64:693–702
36. Hiscox S, Morgan L, Green T et al (2006) Src as a therapeutic target in anti-hormone/anti-growth factor-resistant breast cancer. Endocr Relat Cancer 13(Suppl 1):S53–S59
37. Myoui A, Nishimura R, Williams PJ et al (2003) c-SRC tyrosine kinase activity is associated with tumor colonization in bone and lung in an animal model of human breast cancer metastasis. Cancer Res 63:5028–5033
38. Rucci N, Recchia I, Angelucci A et al (2006) Inhibition of protein kinase c-Src reduces the incidence of breast cancer metastases and increases survival in mice: implications for therapy. J Pharmacol Exp Ther 318:161–172
39. Hussar DA (2007) New drugs: paliperidone, dasatinib, and decitabine. J Am Pharm Assoc 47:298–302
40. Boyce BF, Xing L, Shakespeare W et al (2003) Regulation of bone remodeling and emerging breakthrough drugs for osteoporosis and osteolytic bone metastases. Kidney Int Suppl 85:S2–S5
41. Knerr K, Ackermann K, Neidhart T et al (2004) Bone metastasis: osteoblasts affect growth and adhesion regulons in prostate tumor cells and provoke osteomimicry. Int J Cancer 111:152–159
42. Chung LW, Huang WC, Sung SY et al (2006) Stromal-epithelial interaction in prostate cancer progression. Clin Genitourin Cancer 5:162–170
43. Pratap J, Javed A, Languino LR et al (2005) The Runx2 osteogenic transcription factor regulates matrix metalloproteinase 9 in bone metastatic cancer cells and controls cell invasion. Mol Cell Biol 25:8581–8591
44. Barnes GL, Javed A, Waller SM et al (2003) Osteoblast-related transcription factors Runx2 (Cbfa1/AML3) and MSX2 mediate the expression of bone sialoprotein in human metastatic breast cancer cells. Cancer Res 63:2631–2637
45. Desai B, Rogers MJ, Chellaiah MA (2007) Mechanisms of osteopontin and CD44 as metastatic principles in prostate cancer cells. Mol Cancer 6:18

46. Huang WC, Xie Z, Konaka H et al (2005) Human osteocalcin and bone sialoprotein mediating osteomimicry of prostate cancer cells: role of cAMP-dependent protein kinase A signaling pathway. Cancer Res 65:2303–2313
47. Campo McKnight DA, Sosnoski DM, Koblinski JE et al (2006) Roles of osteonectin in the migration of breast cancer cells into bone. J Cell Biochem 97:288–302
48. Adwan H, Bäuerle TJ, Berger MR (2004) Downregulation of osteopontin and bone sialoprotein II is related to reduced colony formation and metastasis formation of MDA-MB-231 human breast cancer cells. Cancer Gene Ther 11:109
49. Minn AJ, Kang Y, Serganova I et al (2005) Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest 115:44–55
50. Javed A, Barnes GL, Pratap J et al (2005) Impaired intranuclear trafficking of Runx2 (AML3/CBFA1) transcription factors in breast cancer cells inhibits osteolysis in vivo. Proc Natl Acad Sci USA 102:1454–1459
51. Bellahcène A, Bachelier R, Detry C et al (2007) Transcriptome analysis reveals an osteoblast-like phenotype for human osteotropic breast cancer cells. Breast Cancer Res Treat 101:135–148
52. Littlewood-Evans AJ, Bilbe G, Bowler WB et al (1997) The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma. Cancer Res 57:5386–5390
53. Le Gall C, Bellahcène A, Bonnelye E et al (2007) A cathepsin K inhibitor reduces breast cancer-induced osteolysis and skeletal tumor burden. Cancer Res 67:9894–9902
54. Morrissey C, Vessella RL (2007) The role of tumor microenvironment in prostate cancer bone metastasis. J Cell Biochem 101:873–886
55. Raisz LG (2005) Pathogenesis of osteoporosis: concepts, conflicts, prospects. J Clin Invest 115:3318–3325
56. Hadjidakis DJ, Androulakis II (2006) Bone remodeling. Ann N Y Acad Sci 1092:385–396
57. Zaidi M (2007) Skeletal remodeling in health and disease. Nature Med 13:791–801
58. Takayanagi H (2007) Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol 7:292–304
59. Varghese S (2006) Matrix metalloproteinases and their inhibitors in bone: an overview of regulation and functions. Front Biosci 11:2949–2966
60. Kollet O, Dar A, Lapidot T (2007) The multiple roles of osteoclasts in host defense: bone remodeling and hematopoietic stem cell mobilization. Annu Rev Immunol 25:51–69
61. Aguila HL, Rowe DW (2005) Skeletal development, bone remodeling, and hematopoiesis. Immunol Rev 208:7–18
62. Paget S (1889) The distribution of secondary growths in cancer of the breast. Lancet 1:571–573
63. Fidler IJ (2003) The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer 3:453–458
64. Schwaninger R, Rentsch CA, Wetterwald A et al (2007) Lack of noggin expression by cancer cells is a determinant of the osteoblast response in bone metastases. Am J Pathol 170:160–175
65. Guise TA, Yin JJ, Mohammad KS (2003) Role of endothelin-1 in osteoblastic bone metastases. Cancer 97:779–784
66. Carducci MA, Jimeno A (2006) Targeting bone metastasis in prostate cancer with endothelin receptor antagonists. Clin Cancer Res 12:6296s–6300s
67. Chaffer CL, Thompson EW, Williams ED (2007) Mesenchymal to epithelial transition in development and disease. Cells Tissues Organs 185:7–19
68. Li L, Neaves WB (2006) Normal stem cells and cancer stem cells: the niche matters. Cancer Res 66:4553–4557
69. Liu S, Dontu G, Wicha MS (2005) Mammary stem cells, self renewal pathways, and carcinogenesis. Breast Cancer Res 7:86–95
70. Wicha MS, Liu S, Dontu G (2006) Cancer stem cells: an old idea-a paradigm shift. Cancer Res 66:1883–1890
71. Bapat SA (2007) Evolution of cancer stem cells. Semin Cancer Biol 17:204–213
72. Felsher DW (2006) Tumor dormancy. Cell cycle 5:1808–1811
73. Karrison TG, Ferguson DJ, Meier P (1999) Dormancy of mammary carcinoma after mastectomy. J Natl Cancer Inst 91:80–85
74. Cameron DM, Schmidt EE, Kerkvliet N et al (2000) Temporal progression of metastasis in lung: cell survival, dormancy and location dependence of metastatic inefficiency. Cancer Res 60:2541–2546
75. Marches R, Scheuermann R, Uhr J (2006) Cancer dormancy. From mice to man. Cell Cycle 5:1772–1778
76. Naumov GN, MacDonald IC, Chambers AF et al (2001) Solitary cancer cells as a possible source of tumour dormancy? Cancer Biol 11:271–276
77. Calvi LM, Adams GB, Weibrecht KW et al (2003) Osteoblastic cells regulate the hematopoietic stem cell niche. Nature 425:841–846
78. Yin T, Li L (2006) The stem cell niche in bone. J Clin Invest 116:1195–1201
79. Lu H, Ouyang W, Huang C (2006) Inflammation, a key event in cancer development. Mol Cancer Res 4:221–233
80. Yoshimura A (2006) Signal transduction of inflammation cytokines and tumor development. Cancer Sci 97:439–447
81. Hiraga T, Myoui A, Choi ME et al (2006) Stimulation of cyclooxygenase-2 expression by bone-dervided transforming growth factor-beta enhances bone metastases in breast cancer. Cancer Res 66:2067–2073
82. Sarkar FH, Adsule S, Li Y, Padhye S (2007) Back to the future: COX-2 inhibitors for chemoprevention and cancer therapy. Mini Rev Med Chem 7:599–608
83. Morony S, Capparelli C, Sarosi I et al (2001) Osteoprotegerin inhibits osteolysis and decreases skeletal tumor burden in syngeneic and nude mouse models of experimental bone metastasis. Cancer Res 61:4432–4436
84. Body JJ, Facon T, Coleman RE et al (2006) A study of the biological receptor activator of nuclear factor-kappaB ligand inhibitor, denosumab, in patients with multiple myeloma or bone metastases from breast cancer. Clin Cancer Res 12:1221–1228
85. Lipton A, Steger GG, Figueroa J et al (2007) Randomized active-controlled phase II Study Of Denosumab Efficacy And Safety In Patients With Breast Cancer-Related Bone Metastases. J Clin Oncol Sep 4; [Epub ahead of print]
86. Tsuchida K, Sunada Y, Noji S et al (2006) Inhibitors of the TGF-beta superfamily and their clinical applications. Mini Rev Med Chem 6:1255–1261
87. Ehata S, Hanyu A, Fujime M et al (2007) Ki26894, a novel transforming growth factor-beta type I receptor kinase inhibitor, inhibits in vitro invasion and in vivo bone metastasis of a human breast cancer cell line. Cancer Sci 98:127–133
88. Eichhorn ME, Kleespies A, Angele MK et al (2007) Angiogenesis in cancer: molecular mechanisms, clinical impact. Langenbecks Arch Surg 392:371–379
89. Kitagawa Y, Dai J, Zhang J et al (2005) Vascular endothelial growth factor contributes to prostate cancer-mediated osteoblastic activity. Cancer Res 65:10921–10929
90. Folkins C, Man S, Xu P et al (2007) Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. Cancer Res 67:3560–3564
91. Thakkar SG, Choueiri TK, Garcia JA (2006) Endothelin receptor antagonists: rationale, clinical development, and role in prostate cancer therapeutics. Curr Oncol Rep 8:108–113