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Mechanisms of bone invasion and metastasis in human neuroblastoma

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Abstract

Bone is the second most common site of metastasis in neuroblastoma. Over the last several years, our understanding of the mechanism of bone metastasis in neuroblastoma has significantly improved. Like breast cancer and myeloma, neuroblastoma cells activate osteoclasts to form osteolytic lesions. Activation occurs via the receptor activator of NFκB ligand (RANKL) or in the absence of RANKL via activation of bone marrow mesenchymal stem cells and stimulation by these cells of the expression of IL-6, a potent osteoclast activating factor. Several targets for therapeutic intervention can now be identified. Inhibition of osteoclast activation by bisphosphonates has already shown to be effective in preclinical models of neuroblastoma bone metastasis and should now be tested in phase I clinical studies. Inhibition of RANKL and IL-6 are other potential targets that require preclinical studies before being tested in patients. This article provides a review of our current understanding of the mechanisms involved in bone metastasis in neuroblastoma and discusses how this knowledge is leading to the identification of new targets for therapeutic intervention.

Keywords: Neuroblastoma; Bone metastasis; Bone marrow mesenchymal stem cells; Bisphosphonates; IL-6

1. Introduction

Bone metastasis is the second most common site of metastasis in neuroblastoma and is observed in 56% of the cases of metastatic neuroblastoma [1]. The mechanisms by which neuroblastoma cells invade the bone have, however, only begun to be elucidated. In this article, a review of our most recent knowledge of the mechanisms of bone invasion and metastasis in

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neuroblastoma is presented, and how this knowledge is allowing identification of novel therapeutic targets is discussed.

2. Bone metastasis

The establishment of bone metastasis is the result of a close interaction between metastatic tumor cells and the unique environment of the bone and bone marrow [2]. Our understanding of these interactions has substantially improved over the last decade and specific pathways responsible for bone metastasis have been identified. In order to form bone metastasis, tumor cells need to leave their primary environment and establish themselves in the bone marrow [3]. This multiple step process involves invasion of the surrounding tissue, penetration of blood vessels (intravasation) and arrest in the bone marrow cavity. Tumor cells use blood vessels that colonize the primary tumor as a result of angiogenesis to have access to the blood circulation, and acquire protease-dependent and -independent movement to penetrate tissue [4]. However, in many cases, the presence of circulating tumor cells in the peripheral blood does not imply the presence of metastasis and establishment of tumor cells at a distant site is usually dependent on organ-specific factors that provide a favorable soil. This 'seed and soil' theory was first proposed by Stephen Paget in 1889, and is now supported by many preclinical models [5]. In this regard, the bone is a particularly fertile soil for tumor cells because, it is a large repository of immobilized growth factors such as transforming growth factor β (TGFβ), insulin-like growth factors I and II, fibroblast growth factor, platelet-derived growth factor, and bone morphogenic proteins which when released upon bone degradation become activated and stimulate tumor cell and osteoblast proliferation and release of parathyroid hormone related peptide (PTHrP). Circulating tumor cells are actively recruited to the bone marrow and the bone. Breast cancer cells expressing the chemokine receptor CXCR4 are, for example, specifically attracted to the bone marrow space by stromal derived factor (SDF-1), a CXC chemokine secreted by bone marrow stromal cells [6]. Human SH-SY5Y neuroblastoma cells also express CXCR4 and human recombinant SDF-1 and bone marrow-derived constituents induce the migration of these cells, as well as their adhesion to bone marrow stromal cells and their proliferation [7]. These data, thus suggest that the CXCR4/SDF-1 pathway plays an important role in the homing of neuroblastoma cells to the bone marrow space. This concept was further supported by the recent observation that CXCR4 is expressed in primary neuroblastoma tumor samples and that higher levels of expression correlate with high-stage disease and the presence of bone and bone marrow metastasis [8].

3. Bone invasion

The bone is a tissue highly resistant to destruction. It is composed of type I collagen and minerals in the form of calcium phosphate. The bone is the subject of constant remodeling through the combined and coordinated activities of two specialized cells, bone building osteoblasts and bone destroying osteoclasts [9]. Osteoblasts are of mesenchymal origin and produce collagen. Osteoclasts are of hematopoietic origin (monocyte-macrophages) and are specialized cells that actively degrade the bone matrix. Osteoblasts and osteoclasts cooperate in maintaining the homeostasis of the bone tissue. In response to stimulation by parathyroid hormone (PTH) osteoblasts produce the ligand for the receptor activator of NFκB (RANK) which is essential for osteoclast differentiation. Osteoclast precursor cells express RANK and upon its activation, NFκB up-regulates the expression of several genes like cathepsin K, matrix metalloproteinase-9, H^+-ATPase, which are responsible for osteoclast maturation and activity. Adhesion of osteoclasts to the bone matrix via the integrin $\alpha_v\beta_3$ and the release into an acidic LAGUNA of lysosomal enzymes like cathepsin K allows the degradation of bone collagen after decalcification by the acidic microenvironment. The interaction between RANKL and RANK is regulated by osteoprotegerin (OPG), a member of the tumor necrosis (TNF) receptor family produced by osteoblast and bone marrow stromal cells. OPG acts as a decoy receptor for RANKL by binding to RANKL and preventing interaction with its receptor. This interaction results in inhibition of osteoclast differentiation, activation and survival [10]. Most of our

knowledge of the mechanisms of bone invasion has been derived primarily from studies in breast cancer, multiple myeloma and prostate cancer which frequently metastasize to the bone [11–13]. Bone metastasis in cancer is arbitrarily classified into osteoclastic and osteoblastic on the basis of the type of cells that are predominantly activated, although in most cases a combination of osteoblastic and osteoclastic activities is seen [14]. In osteoblastic bone metastasis, the feature of prostate cancer, there is a significant amount of disorganized bone formation. In osteoclastic bone metastasis typically observed in breast cancer, multiple myeloma and thyroid cancers, a dramatic increase in the activation of osteoclasts and a substantial amount of bone degradation are typically observed. Activation of osteoclasts by tumor cells is initiated upon adhesive interaction between tumor cells and bone marrow-derived cells, which triggers the production of PTHrP by tumor cells (Fig. 1) [15]. PTHrP stimulates the expression of RANKL in osteoblasts, which promotes the differentiation of osteoclast precursor cells and their maturation into active osteoclasts. However, tumor cells can also bypass the PTHrP-dependent pathway and the need for osteoblasts by producing osteoclast activating factors (OAF) like IL-1β, IL-6, IL-11, macrophage inflammatory protein-1α (MIP-1α), TNFα and RANKL. Osteoclasts express the ubiquitous gp130 receptor (the β receptor) which forms tetra and hexameric complexes with a series of receptors (the α receptor) of the interleukin-6 (IL-6) family of cytokines that includes IL-6, IL-11, oncostatin M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor and cardiotrophin-1. The presence of these 2 receptors at the surface of osteoclasts make them strongly responsive to IL-6 stimulation [16].

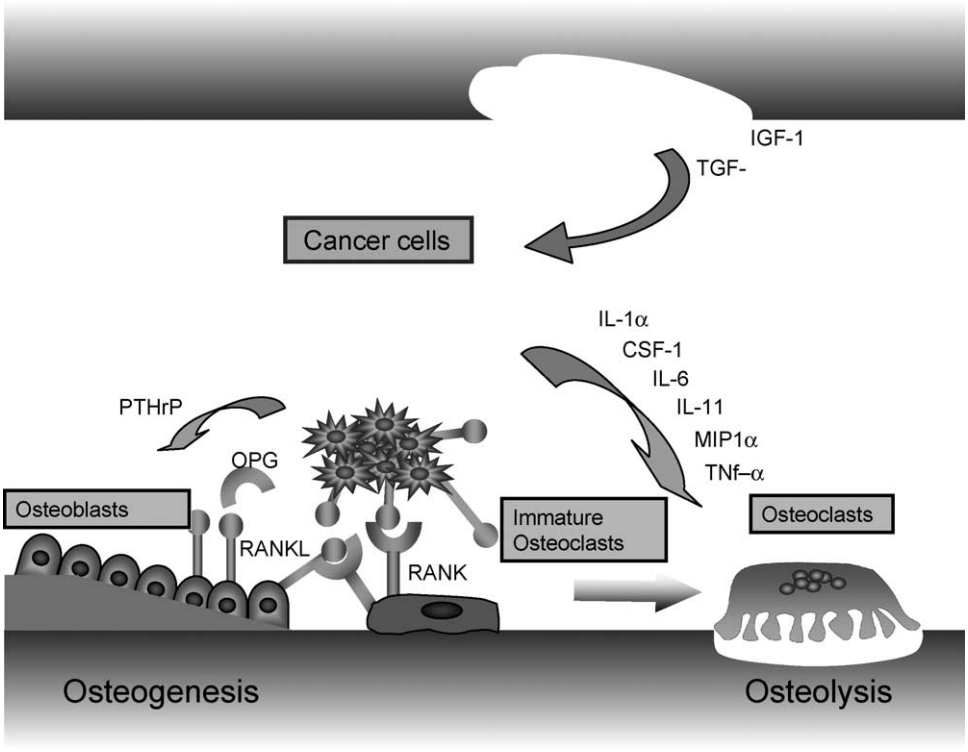

Fig. 1. The vicious circle of bone metastasis. Tumor cells stimulate osteoclast differentiation and activity by (1) producing PTHrP which increases the expression of RANKL in osteoblasts, (2) directly expressing RANKL, and (3) secreting osteoclast activating factors like IL-1α, CSF1, IL-6, IL-11, MIP1α, or TNFα. As a result of bone degradation, growth factors like TGFβ and IGF-1 are released from the bone matrix and further stimulate tumor cell and osteoblast proliferation.

Until recently, little was known of the mechanisms of bone invasion in neuroblastoma. That this, process involved osteoclasts was suggested by studies demonstrating that a human neuroblastoma cell line NB19 stimulated osteoclast activation when cocultured in the presence of osteoclast precursor cells and stromal cells, and that this stimulation was associated with an increased expression of RANKL [17]. In our laboratory, using an in vivo xenotransplantation model in which human neuroblastoma cells were injected directly into the femoral bone marrow space of nu/nu mice, we observed a dramatic increase in osteoclasts as mice developed radiologically detectable osteolytic lesions in the femur. Further, supporting an active role for osteoclasts, we demonstrated that in this model administration of ibandronate, a bisphosphonate inhibitor of osteoclast activity, prevented the formation of osteolytic lesions [18]. A major mechanism of osteoclast activation by neuroblastoma is via the production of RANKL. Several human neuroblastoma cells have been shown to express high levels of RANKL and low levels of OPG. Activation of osteoclasts by these cells can be blocked by OPG, neutralizing antibodies against RANKL or antisense oligonucleotides [19]. However, many neuroblastoma cell lines that form bone metastasis or invade the bone do not express RANKL or other OAF [20]. In vitro experiments demonstrated that although these neuroblastoma cells could not activate osteoclasts when cultured in the presence of osteoclast precursor cells, activation did occur upon addition of bone marrow mesenchymal stem cells. It was then discovered that in the presence of neuroblastoma cells, bone marrow mesenchymal stem cells secrete large amounts of IL-6. Furthermore, activation of bone marrow mesenchymal stem cells by neuroblastoma cells did not require cell–cell contact and could be achieved in the presence of serum free conditioned medium from neuroblastoma cells. There are thus, multiple pathways by which neuroblastoma cells can activate osteoclasts (Fig. 2). Whereas the expression of

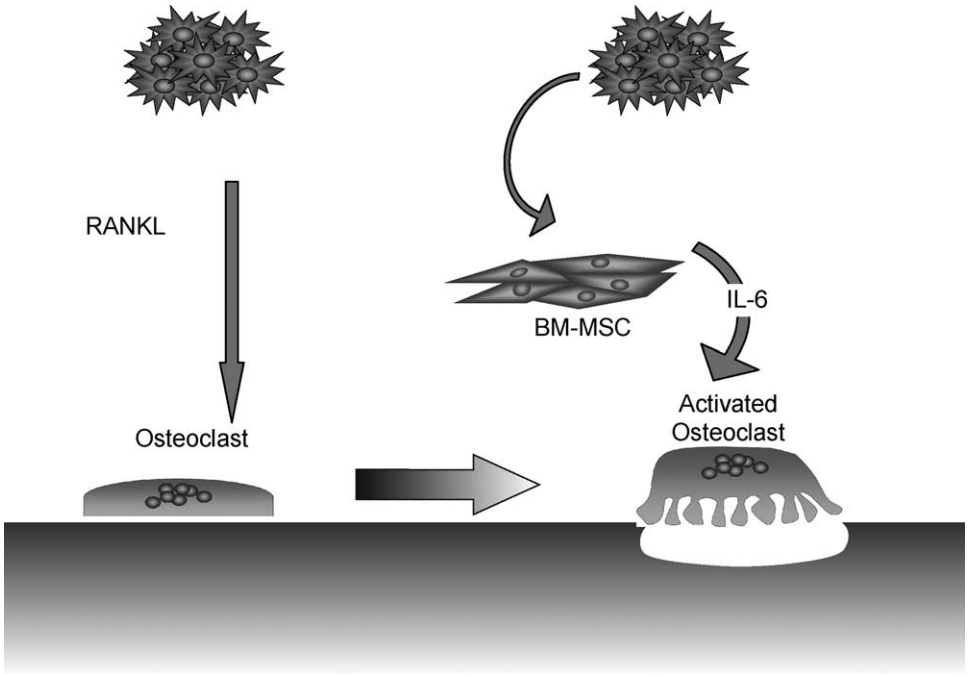

Fig. 2. Multiple pathways for activation of osteoclasts by neuroblastoma cells. Left: several neuroblastoma cells express RANKL, which directly activates osteoclasts. Right: in the absence of RANKL, neuroblastoma cells can stimulate bone marrow mesenchymal stem cells to express IL-6.

RANKL or stimulation of RANKL expression by osteoblasts occurs in some neuroblastoma cells, other neuroblastoma cell lines that do not express RANKL and other OAF activate osteoclasts by indirectly up-regulating the expression of IL-6 in bone marrow mesenchymal stem cells. The presence of these multiple pathways of osteoclast activation may in part explain the high rate of bone invasion in neuroblastoma.

4. Preclinical models to study bone metastasis and invasion in neuroblastoma

The availability of reliable animal models to study bone metastasis in neuroblastoma is essential to test novel therapeutic approaches in preclinical studies. Systemic injections of tumor cells in immunodeficient mice by either intravenous or intracardiac routes result in the formation of osteolytic lesions that can be radiologically detected [21]. These lesions commonly occur in the distal femur or proximal tibia. These models test the ability of tumor cells to locate to the bone when already present in the blood circulation. However, many neuroblastoma cell lines do not form osteolytic lesions when injected systemically. Orthotopic implantation of neuroblastoma cells or small tumor explants more closely mimics the natural behavior of the cancer and is associated with metastasis to the liver, bone marrow, lungs, lymph nodes, ovaries and bone [22,23]. However, this model has the disadvantage that often mice die from distant metastasis in other organs than the bone, so that they are not suitable to study bone invasion. For this particular reason, we have developed in our laboratory a bone invasion model by injecting 2×10^5 neuroblastoma cells into the bone marrow of the femur of nu/nu mice. In this model, several neuroblastoma cell lines created osteolytic lesions that could be easily detected by high resolution X-ray (Faxitron equipment) within 3–8 weeks. A grading system assessing the severity of these radiological lesions was established and used as a clinical end point to establish Kaplan Meier regression curves evaluating response to therapy [20].

5. Toward novel treatment for bone metastasis in neuroblastoma

A better understanding of the mechanisms of bone metastasis and invasion in neuroblastoma and the availability of reproducible animal models allow for testing novel therapeutic pathways more specifically directed towards bone metastasis in neuroblastoma. A first target is osteoclasts since, it has now been clearly demonstrated that bone metastasis in neuroblastoma is primarily an osteolytic process associated with an increased activation of osteoclasts. Over the last decade several inhibitors of osteoclast activation have been developed. Among the most effective agents are bisphosphonates, metabolically stable analogues of pyrophosphate (P–O–P) in which the central oxygen atom is replaced with a carbon atom (P–C–P). These compounds bind with very high affinity to the hydroxapatite in mineralized bone [24]. The mechanism by which bisphosphonates interfere with osteoclast activation is not entirely understood, however, nitrogen containing bisphosphonates induce cell apoptosis by inhibiting farnesyl diphosphate synthase, a key enzyme in the mevalonate pathway, thereby reducing the prenylation of small GTP-binding proteins that are essential for cell survival [25]. Interestingly, the apoptotic activity of these compounds is not restricted to osteoclasts and includes tumor cells if given at sufficiently high concentrations. Despite this potent cytotoxic effect in the bone, bisphosphonates have surprisingly little toxicity on other organs. This is due to their very high affinity for the bone matrix and their very short plasma half-life. Several bisphosphonates, and in particular zoledronic acid, are currently tested in phase I and II trials in myeloma and in breast and prostate cancers that have metastasized to the bone, with promising results [26]. In our laboratory, we have obtained preclinical evidence that bisphosphonates are effective in preventing the development of osteolytic lesions in mice locally injected with neuroblastoma tumor cells, suggesting, therefore, that these agents could be effective in patients with neuroblastoma metastatic to bone. Interference with RANK–RANKL interaction is another potential therapeutic target. Osteoprotegerin, the natural RANKL decoy receptor has been recently developed in a recombinant form and is currently tested in clinical trials for multiple myeloma

and breast cancer [27]. Evidence of the involvement of RANKL–RANK in some neuroblastoma cell lines suggests that it may be of therapeutic value in bone metastasis in neuroblastoma. Our data also suggest that IL-6 could be a target for therapeutic intervention in neuroblastoma that has metastasized to the bone. Inhibition of IL-6/IL-6R interaction by blocking monoclonal antibodies has been tested in a large number of animal models [28] and is currently considered in the treatment of multiple myeloma where IL-6 has been shown to be the major OAF responsible for bone osteolytic lesions [29]. Alternatively, the proteasome inhibitor PS-341 is one of the most promising novel agents in multiple myeloma. This agent inhibits IL-6 triggered phosphorylation of ERK and the NFκB dependent transcription and secretion of IL-6 [30]. Whether IL-6/IL-6R blocking could be of therapeutic value in neuroblastoma bone metastases will require preclinical studies in the models previously described and has been initiated in our laboratory.

Finally, blocking the CXCR4/SDF-1 pathway may theoretically be a valuable approach to prevent the formation of bone marrow and bone metastases in neuroblastoma if applied at an early stage. Several agents blocking the binding of SDF-1 to its receptor CXCR4 have been developed initially as potential anti-HIV drugs since CXCR4 is also the chemokine co-receptor used by the HIV virus to enter and infect T cells [31]. Among those, AMD 3100 has been shown to have antiviral efficacy [32]. Whether such agents could be valuable in the treatment of neuroblastoma or other cancers relying on CXCR4/SDF-1 to colonize the bone marrow remains to be determined. The safety of AMD 3100 in patients with non-Hodgkin's lymphoma and myeloma has been, however, established [33].

6. Conclusion

Our understanding of the mechanisms of bone metastasis in neuroblastoma has substantially improved over the last several years to the point that several pathways leading to an increase in osteoclast activation have been identified. The identification of these pathways and the availability of reliable preclinical models are now allowing us to test novel therapeutic targets for bone metastasis in neuroblastoma. Phase I clinical trials should now be initiated to test the validity of these approaches in children affected with neuroblastoma bone metastasis.

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