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AUTHOR'S VIEWS

Bad to the bone: B cell acute lymphoblastic leukemia cells mediate bone destruction

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ABSTRACT

Skeletal morbidities continue to cause acute and long-term burdens for B-ALL patients underscoring the need to identify the mechanisms underlying these processes and to develop effective therapies. Our recent findings demonstrated that B-ALL cells isolated at patient diagnosis can cause bone destruction and have identified the receptor activator of nuclear factor κ-B (RANK-RANKL) ligand axis as a critical effector of these effects.

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Precursor B cell acute lymphoblastic leukemia (B-ALL) is a result of abnormal proliferation and expansion of B cell progenitors in the bone marrow. Although the survival rates for children with ALL have improved dramatically over the past several decades, acute and long-term toxicities remain a major concern in disease survivors. One of these complications is skeletal morbidities observed at diagnosis, during chemotherapy treatment, and as a long-term effect after treatment.[1,2] An understanding of the molecular mechanisms underlying bone destruction is needed to implement targeted therapies that block or moderate these effects in ALL patients.

The receptor activator of nuclear factor κ-B RANK-RANKL (ligand)-OPG (osteoprotegerin) pathway is critical for the regulation of physiological bone remodeling. RANKL, encoded by the tumor necrosis factor superfamily member 11 (*TNFSF11*) gene, binds to its cognate receptor RANK that signals the differentiation, activation, and survival of osteoclasts and bone remodeling.[3,4] OPG binds to RANKL, preventing RANKL-RANK interaction, and is critical to maintain bone mass homeostasis by inhibiting RANKL-mediated bone resorption and osteoclastogenesis.[5] This pathway is central to pathological bone loss in postmenopausal osteoporosis, multiple myeloma, and solid tumor metastases to the bone.[6] Additional cancer cell-induced signals may further alter the balance between RANKL (activating) and OPG (blocking) effects on RANK-receptor signaling, potentiating osteoclastogenesis, and bone destruction.[7] Tumor-induced osteoclasts enhance bone resorption and contribute to the growth, proliferation, and survival of tumors by releasing growth factors from the bone niche, thereby forming a "vicious cycle."[6,7] Thus, the RANK-RANKL signaling in bone microenvironment plays a critical role in bone destruction in multiple diseases.

We recently reported mechanistic evidence that primary B-ALL cells confer bone destruction in a RANK-RANKL—dependent fashion.[8] Using a mouse model of spontaneous B-ALL,[9] and B-ALL patient-derived xenograft (PDX) models, we demonstrated that both the mouse and human leukemic cells caused bone destruction in the absence of corticosteroids or other chemotherapeutic agents.[8] We showed that RANKL, the critical regulator of bone resorption, was abundantly expressed by the mouse leukemic cells.[8] Transfer of these RANKL-expressing leukemic cells into immunodeficient recipient mice produced extensive vertebral and long bone destruction suggesting that RANKL-expression by the leukemic cells may have contributed to bone loss.[8] Since osteoporosis-associated fractures are evident in 16% of children and adolescent ALL patients at diagnosis,[2] we asked whether human B-ALL cells could also cause bone destruction. Primary B-ALL cells isolated from patients at diagnosis were injected into the femurs of NOD.*Prkdc*$^{scid/scid}$*Il2rg* ^{tm1Wjl}/SzJ (NSG) mice. In this PDX setting, the human B-ALL cells displayed up-regulation of RANKL in the bone microenvironment and conferred both long bone and vertebral trabecular bone destruction[8] (Figure 1). Treatment of PDX recipient mice with the RANKL antagonist recombinant OPG-Fc (rOPG-Fc) conferred robust protection from bone destruction (Figure 1). This protective effect was evident even in the absence of companion chemotherapy treatment allowing unopposed leukemia cell proliferation in the PDX mice.[8] These results provided evidence that RANKL expressed on B-ALL cells is a critical mediator of bone destruction and that blockade of this signaling axis protects the bone even under conditions of heavy disease burden.

Previous mouse models of solid tumor metastasis to the bone have shown that therapeutic RANKL blockade reduced skeletal tumor burden suggesting a dependence on bone resorption and attendant factors released by the bone microenvironment.[7] Upon injection into the femur shaft of PDX recipient mice, we found that primary human B-ALL cells migrated to the metaphyseal region of the growth plate,

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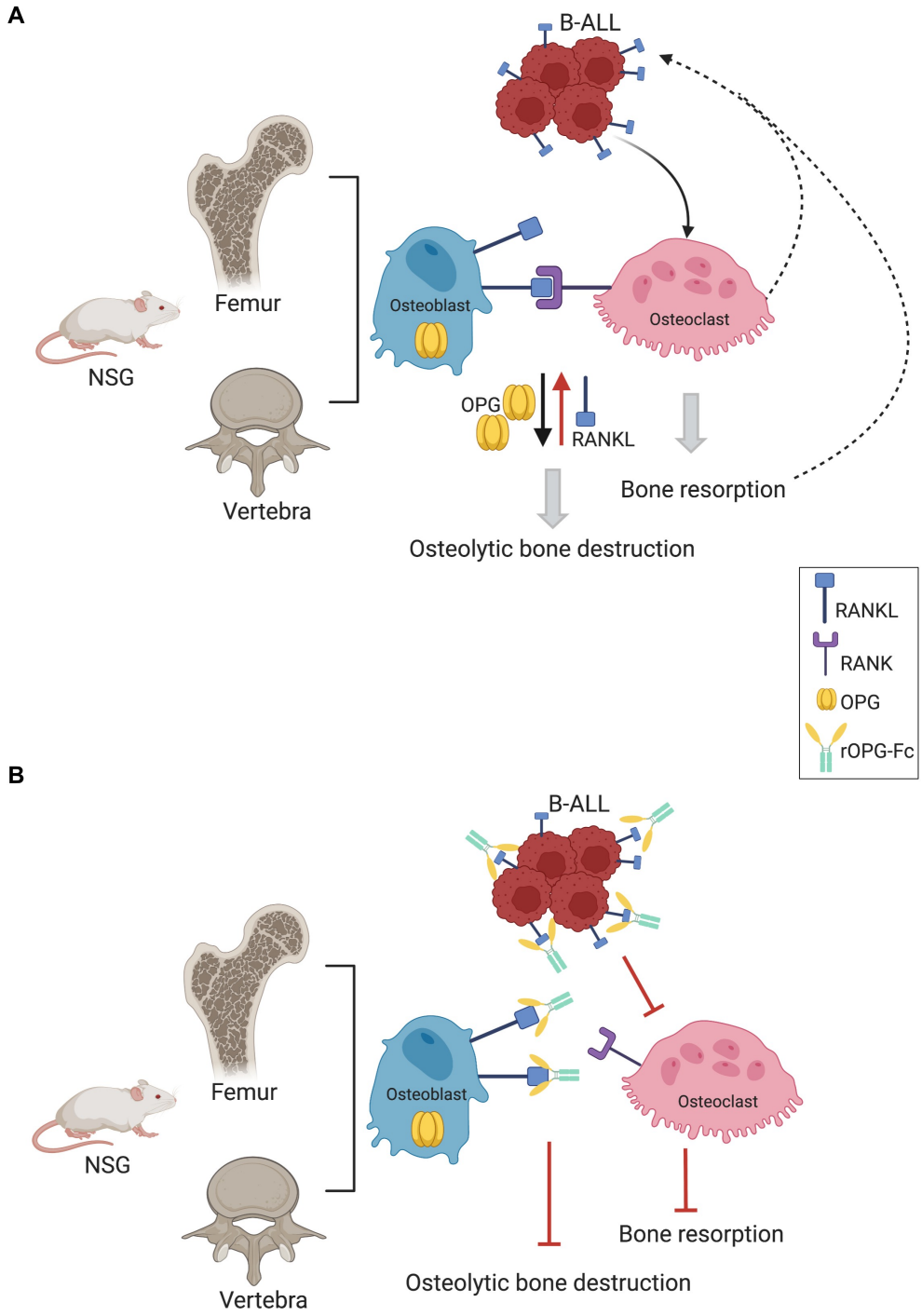

Figure 1. RANKL-mediated bone destruction in a patient-derived xenograft (PDX) model of human B cell acute lymphoblastic leukemia (B-ALL). Schematic representation of receptor activator of nuclear factor κ-B ligand (RANKL) antagonism in the long bones and vertebra of human primary B-ALL cells transplanted NOD.*Prkdc*$^{scid/scid}$*Il2rg* ^{tm1Wjl}/SzJ (NSG) mice. (a) Osteoblast precursors in the mouse and human bone marrow express RANKL, which binds to its cognate receptor activator of nuclear factor κ-B (RANK) on osteoclast precursors. The RANKL-RANK binding initiates downstream signaling within the osteoclast precursors, and initiates the differentiation, activation of osteoclasts resulting in bone resorption. A decrease in osteoprotegerin (OPG), a RANKL soluble decoy receptor, and upregulation of RANKL in the bone marrow niche lead to osteolytic bone destruction. B-ALL cells express RANKL that binds to RANK on osteoclast precursors, acts upon osteoblasts or bone marrow stromal cells, increases RANKL abundance, and further increases bone resorption in specific niches. Increased bone resorption resulting from RANKL-RANK interaction could potentiate release of various growth factors from the bone microenvironment, which could further support leukemic cell survival. (b) In a PDX setting, we found that recombinant OPG-Fc (rOPG-Fc) bound to RANKL on human B-ALL cells and mouse osteoblasts or bone marrow stromal cells, disrupted RANKL-RANK interaction, reduced bone resorption, and attenuated B-ALL mediated osteolytic bone destruction.

where they enhanced bone resorption and growth plate destruction.[8] These data suggest that B-ALL cells interact and exploit specific oxygen- and nutrient-rich niches in this specialized bone microenvironment, which may favor their survival.

For older children and adolescents diagnosed with B-ALL, destruction of their long bone growth plates at a critical time in their maturation compromises their ability to achieve their full adult height. The standard of care for B-ALL patients is intensified multi-agent chemotherapy, including corticosteroids, which

independently confers a significant risk of systemic skeletal osteoporosis and osteonecrosis. Therefore, blockade of both the B-ALL cell- and chemotherapy-dependent effects may be most efficient by co-administration of bone-protective drugs with multi-agent chemotherapy during B-ALL treatment. The critical clinical question to be addressed in controlled trials is whether contemporaneous administration of bone-protective agents compromises the high efficacy of current chemotherapy protocols to cure B-ALL. From a mechanistic perspective, additional studies are needed to determine whether protection of the bone with RANK-RANKL antagonists may alter B-ALL relapse that may arise from leukemic cell sanctuary in specific bone niches.

There is an unmet clinical need to block the skeletal complications of B-ALL particularly in children and adolescents, which could be addressed with a combination of multi-agent chemotherapy and bone-protective targeted therapy. Therapeutic targeting of osteoclasts to prevent tumor-associated bone destruction is an area of intense clinical investigation for multiple myeloma, as well as breast and prostate cancer bone metastasis. Denosumab, an anti-RANKL antibody, is approved to treat these indications.[10] Compared to bisphosphonates such as pamidronate or zoledronic acid, denosumab is well tolerated and with low rates of adverse complications.[10] Our studies suggest that denosumab or other targeted agents that inhibit the RANK-RANKL interaction may be efficacious in patients with B-ALL to reduce the pain, fractures, and long-term effects on the bones in the high proportion of patients who survive rigorous treatment.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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