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HIF targets in bone remodeling and metastatic disease

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ABSTRACT

The bone marrow is a hypoxic microenvironment that is rich in growth factors and blood vessels and is readily colonized by tumor cells disseminated from numerous cancers including tumors of the breast, prostate, lung, and skin. The origin of metastatic growth promoting factors for tumor cells disseminated to the bone marrow is derived from multiple sources: the bone matrix, which is a reservoir for growth factors, and cells residing in the marrow and along bone surfaces, such as osteoblasts, osteoclasts, macrophages, and T cells, which secrete cytokines and chemokines. Low oxygen levels within the bone marrow induce hypoxia signaling pathways such as hypoxia inducible factor (HIF), which is regulated by oxygen requiring prolyl hydroxylases (PHDs) and von Hippel–Lindau (VHL) tumor suppressor. These hypoxia signaling pathways have profound effects on bone development and homeostasis. Likewise, hypoxic conditions observed in local breast and prostate tumors point to a role for hypoxia-inducible genes in metastasis to and colonization of the bone marrow. This review will explore the role of hypoxia-regulated factors in bone development and remodeling, and how these elements may contribute to solid tumor metastasis to the bone.

Contents

1. Introduction

The bone and its associated marrow offer a unique microenvironment to tumor cells with access to growth factors, cytokines, blood supply and tumor-supportive cells including macrophages, T cells and stromal cells. Osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells) play a pivotal role in skeletal development and remodeling, and osteoclasts in particular impact metastatic tumor cells in the bone marrow through resorption of the bone matrix and release of growth factors. Upon dissemination to the bone marrow, cancer cells secrete parathyroid hormone-related protein (PTHrP), which binds to the parathyroid hormone (PTH) type 1 receptor (PTH1R) on osteoblast lineage cells and stimulates production of receptor activator of NFkB (RANK) ligand (RANKL) by osteoblasts (Mundy, 1997; Sterling et al., 2011) (Fig. 1). RANKL binds to its receptor RANK on osteoclast precursors, promoting osteoclast maturation and resorption of the bone matrix. The bone matrix is a reservoir for many cytokines and growth factors, and harbors the largest pool of latent TGF-β1 in the body (Bonewald & Mundy, 1990). Osteoclast resorption of the bone matrix releases TGF-β in its active form and provides a paracrine signal to tumor cells to increase PTHrP production and proliferate, further increasing osteoclast-mediated bone resorption and release of TGF-β (Mundy, 1997; Sterling et al., 2011). This results in osteolytic bone

Abbreviations: BP(s), bisphosphonate(s); CXCL1, -2, -3, -5, -6, -7, -8, -12, C-X-C motif ligand; CXCR1, -2, -4, C-X-C motif receptor; HIF, hypoxia inducible factor; HRE, hypoxia responsive element; MMTV, mouse mammary tumor virus; ONJ, osteonecrosis of the jaw; OS, overall survival; PDGF-BB, platelet-derived growth factor-BB; PDGFRβ, platelet-derived growth factor receptor β; PFS, progression free survival; PHD, prolyl hydroxylase; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; PyMT, polyoma virus middle T antigen; RANK, receptor activator of NFκB; RANKL, receptor activator of NFκB ligand; RCC, renal cell carcinoma; SRE, skeletal-related event; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; VHL, von Hippel–Lindau.

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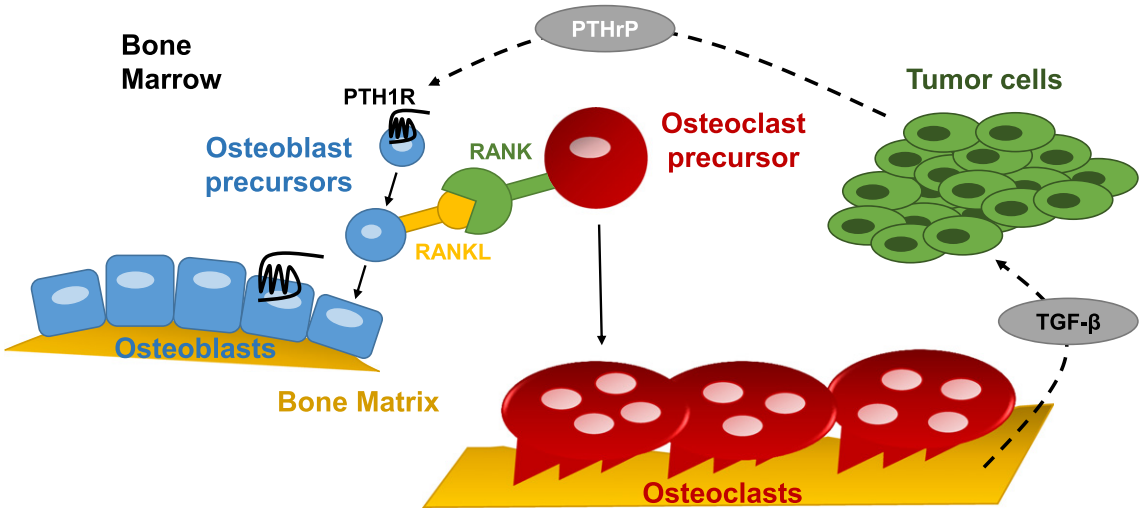

Fig. 1. Mechanisms of tumor-induced bone destruction. Tumor cells disseminated to the bone marrow secrete parathyroid hormone-related protein (PTHrP), which binds to the PTH receptor type 1 (PTH1R) on cells of the osteoblast lineage. PTHrP:PTH1R binding increases receptor activator of NFκ-B (RANK) ligand (RANKL) expression on osteoblasts, which binds RANK on osteoclast precursors to drive osteoclast formation. Osteoclasts resorb the bone matrix releasing active transforming growth factor-β (TGF-β) from the matrix, which stimulates tumor cell proliferation and PTHrP production, thus creating a perpetual cycle of osteolysis.

destruction, which is common in breast and lung cancer bone metastasis, and observed less often in prostate cancer bone metastases. Many of the primary therapies for bone metastatic breast cancer target osteoclasts in an effort to block tumor cell proliferation and osteolytic bone destruction. Melanoma (Patten et al., 1990; Suva et al., 2011), thyroid (Coleman, 2006), breast (Abrams, 1950; Shirazi et al., 1974; Boxer et al., 1989; Coleman, 2006), prostate (Coleman, 2006), and lung (Coleman, 2006; Katakami et al., 2014; Riihimaki et al., 2014) tumors have the highest predilection of solid tumors for the skeleton (Table 1), and renal cell carcinoma (RCC) metastasizes to bone at a frequency of up to 35% (Coleman, 2006; Santini et al., 2013). RCC patients with bone metastases or lung metastases also have a poorer prognosis than patients without bone metastases (McKay et al., 2014; Park et al., 2012). A recent report indicates that metastatic or recurrent gastric cancer also metastasizes to bone at an approximate frequency of 10% (Kim et al., 2014) and was prognostic of significantly shorter overall survival.

The structure of the long bones is such that the proximal and distal ends of the bone (metaphyses) are wider than the mid-shaft (diaphysis) of the bone, and there is a high degree of vascularization in the distal femoral metaphysis and proximal tibial metaphysis. The bone marrow microenvironment harbors strongly hypoxic regions (Parmar et al., 2007; Rankin et al., 2011) and a hypoxia gradient that increases with distance from the metaphysis (Parmar et al., 2007; Kusumbe et al., 2014). This is not surprising given the extent of vascularization present in the metaphyseal region. The diaphysis, in contrast, contains few blood vessels and thus is characterized by immunostaining for nuclear hypoxia inducible factor-1α (HIF-1α) staining and HIF target proteins including MCT4 and Glut1 (Kusumbe et al., 2014). The effect of oxygen tension on vascularity (and lack thereof with regards to the diaphysis) is directly linked to osteogenesis and angiogenesis and the mechanisms that couple these processes.

Table 1
Incidence of bone metastasis by cancer type upon autopsy, except where indicated.

Cancer type	Incidence of bone metastasis
Melanoma	35% (Suva et al. (2011)) 17% (Patten et al. (1990))
Breast cancer	79% (Boxer et al. (1989)) 73% (Coleman (2006); Galasko, 1981) up to 70% (Shirazi et al. (1974); Abrams, 1950)
Prostate cancer	68% (Coleman (2006); Galasko, 1981)
Lung cancer	39% (Riihimaki et al. (2014)) adenocarcinoma, 25% SCLC [48% (Katakami et al. (2014)), NSCLC; 40%, SCLC] at initial diagnosis 36% (Coleman (2006); Galasko, 1981)
Renal cell carcinoma	22% (Santini et al. (2013)) 35% (Coleman (2006); Galasko, 1981)
Gastric cancer	10% (Kim et al. (2014)) 5% (Coleman (2006); Galasko, 1981)
Head and neck cancer	<1% (Bhandari, 2013), squamous cell carcinoma only 12% (León et al., 2000)
Thyroid	42% (Coleman (2006); Galasko, 1981)
Ovarian	0.1% (Suva et al. (2011))

The hypoxia inducible factor (HIF) proteins are the major mediators of the cellular response to low-oxygen, or hypoxic conditions. In normal oxygen conditions (normoxia), HIF-1α and HIF-2α are hydroxylated by a family of prolyl hydroxylases (PHD), consisting of PHD1, -2, and -3. HIFα hydroxylation provides a scaffold for von Hippel–Lindau (VHL) tumor suppressor protein, which results in polyubiquitination and subsequent degradation of the HIF proteins (Huang et al., 1998; Maxwell et al., 1999; Hon et al., 2002). Under hypoxic conditions, PHD activity decreases and the alpha subunits of HIF proteins accumulate, translocate to the nucleus, and bind to hypoxia-responsive elements (HREs) on HIF target genes (Fine & Norman, 2002). Transcriptional activation of hypoxia-responsive genes impacts a number of cell processes that drive tumor cell progression, including angiogenesis and glycolysis.

Oxygen gradients fluctuate in solid tumors, meaning specific regions of the tumor may rapidly transition between high and low oxygen levels (Dewhirst et al., 2008) and may not display uniform oxygen tensions. Mechanistically, this is a consequence of leaky and atypical vasculature that is characteristic of large solid tumors (Shah-Yukich & Nelson, 1988). Hypoxia is associated with poor patient prognosis (Vaupel & Hockel, 2001; Brown & Wilson, 2004; Toustrup et al., 2012) and HIF-1α is increased approximately 3-fold in breast cancer patients who are positive for disseminated tumor cells in bone marrow aspirates (Woelfle et al., 2003), suggesting that the hypoxic conditions the tumor cells encounter in the primary tumor site may promote tumor cell metastasis to bone, and that the hypoxic microenvironment of the bone marrow may promote subsequent bone colonization. This review will discuss the role of HIF and its molecular regulators in bone homeostasis and hypoxic microenvironments with an emphasis on how these processes may contribute to tumor metastasis to and growth in bone. As in other tumor microenvironments, vascularization is pivotal to enable tumor colonization, and the same molecular mediators (VHL, HIF-1α, VEGF) drive these critical processes. Bone remodeling and bone development in particular are in large part fueled by the spatial and temporal