Review

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New therapeutic targets for cancer bone metastasis

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Bone metastases are dejected consequences of many types of tumors including breast, prostate, lung, kidney, and thyroid cancers. This complicated process begins with the successful tumor cell epithelial–mesenchymal transition, escape from the original site, and penetration into the circulation. The homing of tumor cells to the bone depends on both tumor-intrinsic traits and various molecules supplied by the bone metastatic niche. The colonization and growth of cancer cells in the osseous environment, which awaken their dormancy to form micro- and macro-metastasis, involve an intricate interaction between the circulating tumor cells and local bone cells including osteoclasts, osteoblasts, adipocytes, and macrophages. We discuss the most recent advances in the identification of new molecules and novel mechanisms during each step of bone metastasis that may serve as promising therapeutic targets.

Bone metastasis

More than 600 000 cases of cancer bone metastasis are diagnosed every year in the USA [1]. Bone is the third most common site for metastatic disease (after lung and liver) and more prevalent in adult patients (>40 years of age) [2]. Notorious for causing severe pain, bone metastases are also the major reasons for pathologic fracture, life-threatening hypercalcemia, spinal cord compression, immobility, and ultimate mortality in patients afflicted with advanced cancers in the breast, prostate, lung, kidney, thyroid, as well as with hematologic malignancies such as myeloma [3].

Great progress has been made in the medical management of bone metastases including surgery approaches and radiation therapy, as well as targeted medical therapy. However, these strategies are at best only palliative and do not improve overall patient survival [4]. The challenges to identify more effective and specific molecularly targeted therapy to prevent and cure bone metastases as well as enhance the quality of life in these patients remain daunting.

Cancer bone metastasis refers to the ability of cancer cells to leave a primary tumor, travel through circulation, and form a secondary tumor in the distant bone tissue. It comprises five steps: (i) escape from the primary tumor; (ii) invade into lymphatic and/or blood vessels; (iii) survive and travel in the circulation; (iv) land on the bone; and (v) finally flourish in the new bone environment [5,6] (Figure 1). Every step of cancer bone metastasis is tightly regulated by tumor cells, with the cooperation and assistance from non-cancerous cells (Figure 1). In this review, potential new therapeutic targets in every step of cancer metastasis discovered over the past 2 years will be discussed.

Escape from the original site

Epithelial to mesenchymal transition (EMT)

Tumor progression is often facilitated by both intrinsic genetic changes and alterations in the local environment. Although still under debate, increasing evidence suggests that activation of the EMT process is essential to allow carcinoma cells to undergo fundamental cytoskeleton reorganization to lose cohesiveness for single cell migration and escape [7]. Numerous molecules have been reported to be involved in this process. The classical marker of EMT is cadherin switching, in which E-cadherin is lost and N-cadherin is gained [8]. Transcription factors that regulate junctional proteins such as E-cadherin, β-catenin [9] and integrin [10], along with miRNAs and alternative splicing have been reported to play important roles in the EMT [11].

EMT is commonly thought to promote general metastasis; however, Smith *et al*. proposed an interesting hypothesis that EMT activators, that also stimulate the expression of RANKL, a major cytokine for the differentiation of the bone-resorbing osteoclasts from their myeloid progenitors, could drive the tumor cells specifically to the bone. They found that the pro-EMT factor Snail could usher prostate cancer cells to bone and stimulate tumor-bone vicious cycle through calcium and RANKL signaling [12]. Croset and colleagues also reported TWIST1, a well-known EMT-inducing transcription factor, as a contributor to breast cancer bone metastasis through upregulating the expression of miR-10b, a proinvasive factor [13]. By comparing immunohistochemical expression of EMT markers (i.e., E-cadherin, vimentin, NF-κB, Notch-1, ZEB1, and PDGF-D) in prostate cancer specimens (primary and bone metastasis) containing both tumor and adjacent normal tissues, Sethi *et al*. identified Notch-1 as a signature gene for the acquisition of EMT in prostate cancer and its bone metastases [11]. With the advances in profiling methodologies, we expect to see more bone metastasis-specific

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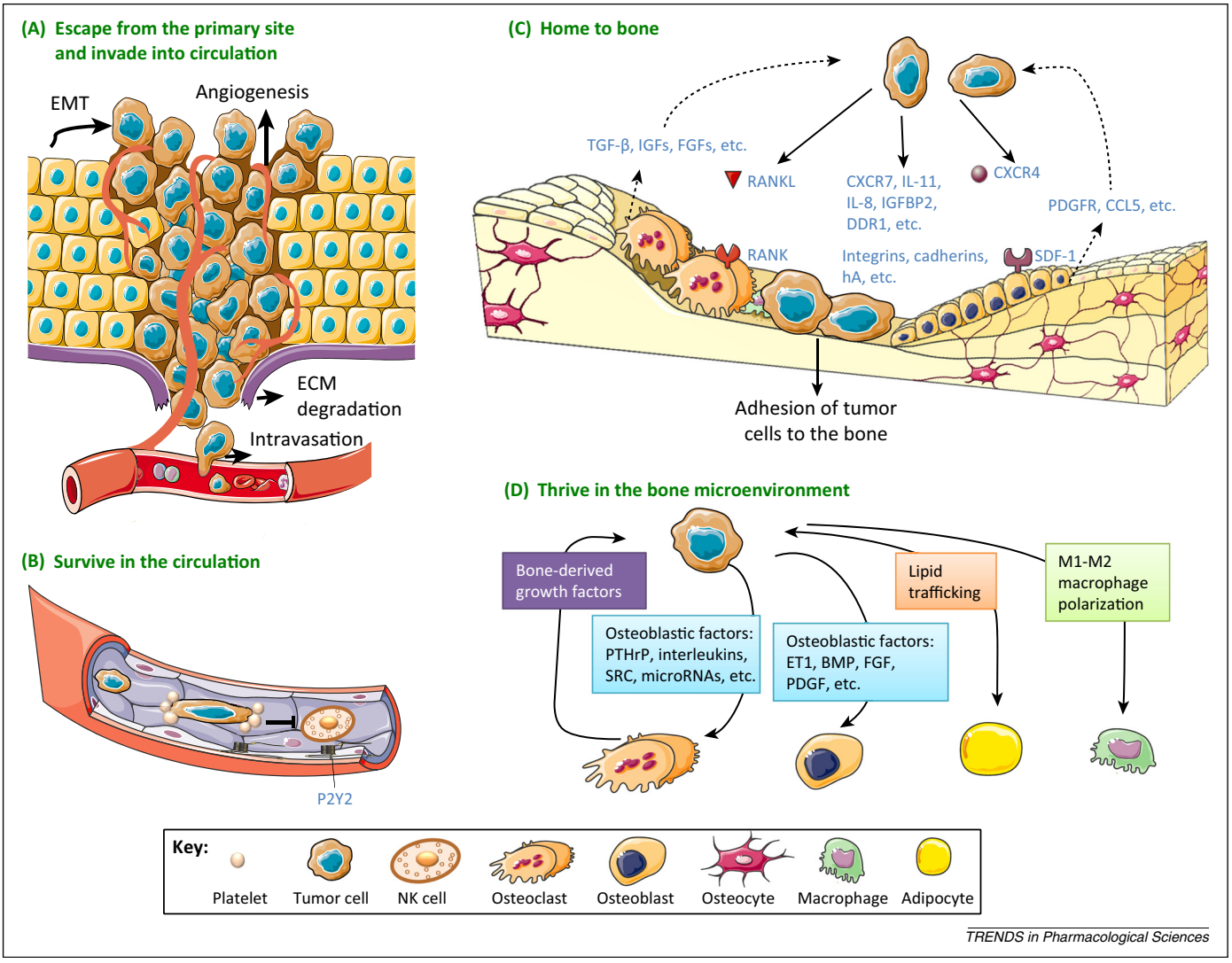

Figure 1. Multiple steps in cancer bone metastasis and the major regulatory factors in the tumor–bone microenvironment. The cellular basis of cancer progression includes tumor cell local invasion, intravasation, survival in the circulation, extravasation, colonization, and proliferation in the bone. In the primary site, tumor cells undergo EMT, ECM degradation, and angiogenesis to invade vascular or lymphatic circulation **(A)**. After intravasation, a few tumor cells can survive from NK cell attack with the assistance of platelets **(B)**. Through communication between tumor-intrinsic factors and factors in the osseous environment, cancer cells extravasate to the bone and marrow. Interactions between tumor cells and bone cells mediated by adhesion molecules are crucial for colonization **(C)**. After landing, tumor cells start to interact with local cells, including osteoclasts, osteoblasts, adipocytes, and macrophages, to thrive in bone and form micro- and macrometastases **(D)**.

targets to be identified during the EMT process, the significance of which awaits functional validations using various molecular genetic and pharmacological approaches.

Hypoxia

Low oxygen level (hypoxia) also increases the malignant behavior of cancer cells, predominantly via hypoxia-inducible factors (HIFs) [14]. Independently of established prognostic parameters, intratumoral hypoxia serves as an adverse indicator for patient prognosis [15]. The activated HIFs induce the expression of pluripotency-associated transcription factors (Oct-3/4, Nanog, and Sox-2) [16], glycolysis- and EMT-associated molecules [17,18], as well as angiogenic factors such as vascular endothelial growth factor (VEGF) [19]. Consequently, targeting the HIF signaling network and the altered metabolic pathways represent a promising strategy to improve the efficacy of current therapies against aggressive and metastatic cancers.

C-X-C chemokine receptor type 4 *(CXCR4)*, osteopontin (OPN), and interleukin-6 and -8 (*IL6* and *IL8*) are hypoxia/HIF-inducible genes in the bone-specific metastatic gene signature in some cell types [20]. In breast cancer cells, HIF-1α and HIF-2α stimulate EMT by potentiating Notch signaling to upregulate SNAIL1 and SLUG – two transcription repressors of E-cadherin [21–23]. Inhibiting HIF-1 activity significantly suppresses breast cancer metastasis to bone in animal models, establishing HIF-1 as a promising therapeutic target [24]. Hypoxia also stabilizes growth arrest-specific 6 (GAS6)/AXL receptor tyrosine kinase signaling in metastatic prostate cancer [25]. Interestingly, transcutaneous CO_2 application not only decreases HIF-1α and increases apoptosis, but also suppresses pulmonary metastases in highly metastatic osteosarcoma cells, suggesting that reoxygenation via a novel transcutaneous CO_2 treatment could be a therapeutic breakthrough for metastasis suppression in osteosarcoma patients [26].