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Endothelial Cells as Precursors for Osteoblasts in the Metastatic Prostate Cancer Bone

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Abstract

Prostate cancer cells metastasize to the bones, causing ectopic bone formation, which results in fractures and pain. The cellular mechanisms underlying new bone production are unknown. In a recent study, Lin and colleagues, by using state-of-the-art techniques, including prostate cancer mouse models in combination with sophisticated *in vivo* lineage-tracing technologies, revealed that endothelial cells form osteoblasts induced by prostate cancer metastasis in the bone. Strikingly, genetic deletion of osteorix protein from endothelial cells affected prostate cancer–induced osteogenesis *in vivo*. Deciphering the osteoblasts origin in the bone microenvironment may result in the development of promising new molecular targets for prostate cancer therapy.

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Prostate cancer is one of the most common types of cancers among male patients around the world, and it is anticipated that its incidence will increase due to the population growth, especially the elderly [1]. The biggest problem that this pathology presents is the expansion of malignant cells to distant organs [2]. Remarkably, prostate cancer cells tend to metastasize in bones, which represent fertile ground for their accommodation and growth [3]. This process results in bone lesions due to tumor cells provoking increased bone formation through osteoblasts activation [4]. This phenomenon is the result of the body's attempt to produce bone repair; however, the result of this growth is weak, aberrantly structured bone tissue [5,6]. Due to the poor quality of the bone produced, patients with this condition suffer higher risk of bone fractures and pain [7]. Additionally, accelerated bone growth produces mineralized tissue containing malignant cells, which, in turn, cause more osteoblastic lesions, creating a vicious cycle of further cancerous growth [8]. Being that new bone accumulation is a critical step in prostate cancer progression, the disruption of osteoblasts generation is a way to decrease tumor burden in the bone. Nevertheless, the cellular and molecular mechanisms that underlie bone production after bone metastases are not completely understood. Deciphering the osteoblasts' origin in the bone microenvironment may result in the development of promising new molecular targets for prostate cancer therapy. (See Fig. 1).

Endothelial cells line the inner surface of blood vessels and play a broad range of roles related to vascular homeostasis [9]. Since these cells have been successfully isolated from a variety of tissues and established in culture, several studies have explored other possible functions for endothelial cells [10]. Evidence suggests that endothelial cells are plastic and may form other cell types, including fibroblasts [11], chondrocytes [12], and osteoblasts [13]. The inherent osteogenic differentiation capacity of endothelial cells was not yet explored in physiologic conditions *in vivo*, and it cannot be discounted that modification of endothelial cells' properties by their manipulation *in vitro* may influence their fate decisions.

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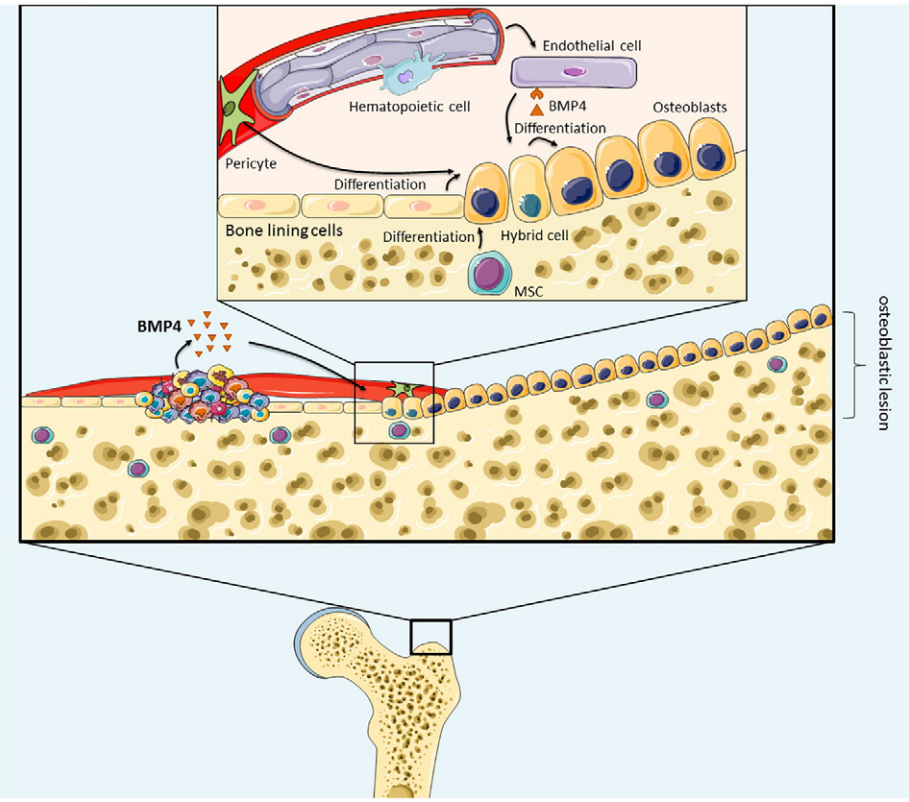

Figure 1. Endothelial cells form osteoblasts in the metastatic prostate cancer bone. Prostate cancer metastases induce ectopic bone formation, which cooperates with prostate cancer progression. Understanding the cellular mechanisms involved in this process is a central question in prostate tumor biology. Lin and colleagues recently showed that endothelial cells, stimulated by malignant cells *via* BMP4, generate osteoblasts [14]. Future studies may reveal the complexity of the bone microenvironment invaded by prostate tumor cells in much greater detail.

In a recent study in *Developmental Cell*, Lin and colleagues showed, by using several mouse models of prostate cancer, *in vivo* gene deletion, and genetic fate-tracing technologies, that endothelial cells generate osteoblasts stimulated by prostate cancer metastasis in the bone [14]. The authors demonstrated that after implantation of patient-derived xenograft PCa-118b, prostate cancer cell line, subcutaneously, mouse-derived cells participate in the ectopic bone formation present in those tumors. Lin and colleagues investigated the progeny of Tie2-expressing cells in these tumors by using Tie2-Cre/TdTomato mice to label specifically endothelial-generated cells. These genetic lineage tracing experiments unveiled that endothelial cells participate in the formation of tumor-induced osteoblasts [14]. Moreover, the authors demonstrated, by the use of osteorix-knockout osteoblasts isolated from the calvaria, that osterix expression is necessary for osteoblast differentiation. Based on this, using state-of-the-art lineage-tracing Cre/loxP-mediated technologies, the authors deleted osteorix protein specifically in endothelial cells. Strikingly, those experiments revealed that osteorix in endothelial cells is essential for prostate cancer–induced osteogenesis *in vivo* [14]. Interestingly, Lin and colleagues detected cells with coexpression of endothelial and osteoblast markers in PCa-118b xenografts, as well as in human bone marrow biopsies of patients with prostate cancer metastasis. These hybrid cells could represent intermediate cells produced in the transition between endothelial cells to osteoblasts. Finally, by the use of viral vectors overexpressing several factors in prostate tumors, the authors suggested that BMP4 induces ectopic bone formation.

Here, we discuss the findings from this work and evaluate recent advances in our understanding of the prostate cancer metastasis bone microenvironment.

Perspectives/Future Directions

Lin and colleagues reveal that, in the metastatic prostate cancer lesions, the bone arises partly from bone marrow endothelial cells. This unpredictable plasticity of endothelial cells to form bone in the bone marrow microenvironment may lead to the design of innovative treatments to inhibit specifically these cells for the improvement in the outcome of patients with bone metastasis.

The main conclusions from this study are based on the data acquired from Tie2-Cre/TdTomato and Tie2-Cre/osteorix floxed mice [14]. Yet, although Tie2 gene is expressed in endothelial cells [15,16], it is known that Tie2 expression is not restricted to these cells, as it is also expressed in hematopoietic cells [17,18]. Thus, Tie2-Cre mice display Cre recombinase activity in both endothelial and hematopoietic cells [19–22]. During embryonic development, endothelial cells and hematopoietic stem cells, which form all hematopoietic cells, arise from the same shared precursor: hemogenic endothelium [23–28]. Due to this, endothelial-specific promoters with constitutively active Cre recombinase are not a great tool to prevent Cre recombinase activity in hematopoietic cells. Therefore, it is possible that the labeled osteoblasts, observed in the ectopic bone induced by prostate cancer by Lin et al. (2017) [14], are derived from hematopoietic cells, which would also be very important. Nonetheless, the clarification of what is the exact origin of osteoblasts in the

metastatic bone is still needed. To achieve endothelial-specific tracking and gene targeting, more specific mouse models should be used in future experiments, such as VE-Cadherin-CreERT2 mice [29]. In VE-Cadherin-CreERT2/TdTomato mice, it is possible to track the endothelium's fate specifically, while in VE-Cadherin-CreERT2/osteorix floxed mice, it will be possible to control osteorix protein expression specifically in endothelial cells temporally.

Furthermore, the authors did not quantify the contribution of Tie2-expressing cells to osteoblasts in the prostatic malignancy-induced bone. Thus, it still needs to be addressed whether bone marrow Tie2-expressing cells are the main source of osteoblasts in the prostate cancer metastatic microenvironment. Also, it remains to be revealed which are the other possible sources of osteoblasts in these conditions. Interestingly, several cell populations with osteogenic capacity have been described in the bone marrow [30,31], including NG2+ pericytes [32–50], LepR+ cells [51,52], and Gli1+ cells [53]. Whether these cellular populations contribute to bone formation in prostate cancer requires further investigations, and what is the exact overlap between these cells with Tie2-expressing cells in their role as sources for osteoblasts remains unknown. Additionally, the roles of all stromal cellular populations and innervations [54] in the tumor-induced ectopic bone formation as compared to physiologic bone development remain unrevealed and should be evaluated in future studies. Importantly, the authors showed that deletion of osteorix in Tie2-expressing cells in mice not bearing prostate cancer does not affect the skeletal phenotype [14], suggesting that Tie2+ cells do not form osteoblasts during normal physiologic bone development.

Lin and colleagues suggest BMP4 as a specific molecule produced by tumor cells that promote osteoblast formation in the metastatic bone [14]. However, whether tumor cells–derived BMP4 is important for this process to occur remains elusive, as BMP4 has not been conditionally deleted from malignant cells or alternative sources. Thus, there is no direct evidence that prostate tumor cells are the only/main functionally important source of BMP4 for ectopic bone formation. BMP4 preferentially binds to TGFβ type I receptors [55], which may be expressed by endothelial cells [56]. Since these receptors have not been conditionally deleted from endothelial cells in the bone, there is also no direct evidence that endothelial cells may be activated to differentiate into osteoblasts through these receptors *in vivo*. Moreover, as the bone prostate tumor microenvironment produces several soluble biologically active factors, future studies should explore whether other molecules produced in this microenvironment *in vivo* may be important in the regulation and/or promotion of new osteoblasts formation in the metastatic bone.

Endothelial cells are not homogeneous in their distribution, morphology, antigen composition, gene expression, and function. These cells vary between different organs, as well as between the various segments of the vasculature within the same organ [57,58]. It remains to be elucidated, for example, whether bone marrow endothelial cells from sinusoids and arteries differ. Also, it is unclear whether Tie2+ endothelial cells are heterogeneous based on their role as a source for osteoblasts in prostate cancer. Does the plasticity of endothelial cells depend on their vascular bed of origin (sinusoid or arteriole)? Is the capacity to form osteoblasts limited to only a specific subpopulation of endothelial cells? Elucidating the molecular differences between endothelial cells in the bone marrow may bring novel concepts about the role of these cells in ectopic bone formation in neoplasic conditions.

A significant limitation in prostate cancer research is the lack of appropriate preclinical models, which allow studying the cellular and molecular processes involved in tumorigenesis. The xenograft and allograft prostate tumor mouse models were used by Lin and colleagues [14]. These models represent great tools to study some aspects of prostate cancer; however, they present limitations related to the immunosuppressed system of the host which is crucial in human metastatic prostate tumor dissemination. Also, the use of cancer cell lines bypasses several primordial stages involved in tumor development, and the interaction with malignant microenvironment may be altered. Thus, it will be interesting to evaluate whether ectopic osteoblast formation occurs in the metastatic bone in genetically engineered mouse models, e.g., C3(1)-Tag [59] or others, in which prostate cancer progression is closely reproduced, representing a better predictive mouse model.

In conclusion, understanding the cellular and molecular processes involved in ectopic bone growth is a central question in the prostate cancer metastatic microenvironment biology. The origin of all osteoblasts that form ectopic bone remains unknown. Lin and colleagues revealed endothelial cells as a source for malignancy-induced osteoblasts in prostate cancer [14]. This new knowledge advances our comprehension of the prostatic cancer microenvironment and may result in the future in the development of promising new molecular targets for prostate cancer therapy.

Disclosures

The authors indicate no potential conflicts of interest.

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