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

Ana E. Paiva*, Luiza Lousado*, Viviani M. Almeida*, Julia P. Andreotti*, Gabryella S.P. Santos*, Patrick O. Azevedo*, Isadora F.G. Sena*, Pedro H.D.M. Prazeres*, Isabella T. Borges*, Vasco Azevedo†, Akiva Mintz‡ and Alexander Birbrair*

*Department of Pathology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; †Department of General Biology of Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; ‡Department of Radiology, Columbia University Medical Center, New York, NY, USA

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.

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.

Address all correspondence to: Alexander Birbrair, PhD, Department of Pathology Institute of Biological Sciences of Federal University of Minas Gerais, Belo Horizonte, Brazil. E-mail: birbrair@ich.ufmg.br

Received 31 July 2017; Revised 20 August 2017; Accepted 22 August 2017

© 2017 The Authors. Published by Elsevier Inc. on behalf of Neoplasia Press, Inc. This is an open access article under the CC-BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). 1476-5586

https://doi.org/10.1016/j.neo.2017.08.007
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 osteorix 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 [14]. 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 bone is an important question that requires further investigation.
Endothelial Cells Form Osteoblasts in the Metastatic Bone

Paiva et al.

Neoplasia Vol. 19, No. 11, 2017

930

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 sinuses 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 have not been conditionally deleted from endothelial cells in the bone marrow may bring novel concepts about the role of these cells in ectopic bone formation in neoplastic 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.

Acknowledgements

Alexander Birbrair is supported by a grant from Pró-reitoria de Pesquisa da Universidade Federal de Minas Gerais (PRPq/UFMG) (Edital 05/2016); Akiva Mintz is supported by the National Institutes of Health (1R01CA179072-01A1) and by the American Cancer Society Mentored Research Scholar grant (124443-MRSG-13-121-01-CDD).
chondrogenic and osteogenic potentials at the clonal level. 
J Orthop Res 31, 1089–1095.

Wylie-Sears J, Aikawa E, Levine RA, Yang JH, and Bischoff J (2011). Mitral valve endocardial cells with osteogenic differentiation potential. 
Arterioscler Thromb Vasc Biol 31, 598–607.

Lin SC, Lee YC, Yu G, Cheng CJ, Zhou X, Chu K, Mursheed M, Le NT, Baseler L, and Ahe J, et al (2017). Endothelial-to-osteoblast conversion generates osteoelastic metastasis of prostate cancer. 
Dev Cell 41, 467–480.

Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, and Papadopoulos N, et al (1997). Angiopoietin-2, a natural antagonist for Tie2 that disrupts vivo angiogenesis.
Science 277, 55–60.

Schurch H and Risau W (1993). Expression of tie-2, a member of a novel family of receptor tyrosine kinases, in the endothelial cell lineage. 
Development 119, 957–968.

Sheng Z, Fung WC, Tischler AS, and Wiltzius JJ, et al (2004). Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. 
Cell 118, 149–161.

Takahara S, Xu H, Tanaka J, Kanzaki Y, Yamada S, Sugiyama K, and Suda T (2004). Tie2 signaling is involved in osteoblastic metastasis of prostate cancer. 
J Orthop Res 22, 324–331.

Kisanuki YY, Hammer RE, Miyazaki J, Williams SC, Richardson JA, and Hammerling G, et al (2001). TIE2+ lineage to primitive and definitive hematopoietic cells. 
Establishment of definitive hematopoiesis. Immunity 9, 677–686.

Ito K, Koyama S, Fujie Y, Nakamura A, Nakamura T, Sekiguchi Y, and Arai R, et al (2007). Pericytes are essential for skeletal muscle regeneration and fat accumulation. 
Stem Cell Rev 3, 143–150.