Altered Myeloid Memory Function by BMP Signaling in Breast Cancer Bone Metastases

Claire L. Ihle1, Desiree M. Straign1, Johana A. Canari2, Kathleen C. Torkko1, Kathryn L. Zolman1, E. Erin Smith1, Philip Owens1, 3
1Department of Pathology, University of Colorado Anschutz Medical Campus, Aurora CO 80045; 2Case Western Reserve University, Cleveland OH 44106; 3Research Service, Department of Veterans Affairs, Denver CO Station 554; Abstract 1448; Correspondence: claire.ihle@cuanschutz.edu

Abstract

Background: Metastatic breast cancer (mBC) patients exhibit a 5-year survival rate of only 20% and metastasis most commonly occurs in the bone. Myeloid components in the bone microenvironment are critical mediators of these tumor responses, resulting in long-lasting inflammatory myeloid memory. Myeloid memory has been shown to promote anti-tumor immunogenic functions in the tumor microenvironment (TME). The TME in bone includes unique myeloid cells which are dynamically regulated by bone morphogenic proteins (BMPs). We investigated myeloid memory in the context of BMP signaling in the mBC bone TME, identifying the driver of myeloid functions distinct to mBC bone metastases will advance the understanding of potential immunotherapies to overcome incurable mBC bone lesions.

Methods: A cohort of human mBC bone metastases and matched patient primary tumors and bone metastases were assembled and profiled. Gene expression analysis was performed on patient bone metastases and matched primary tumors using nCounter gene expression probes. Clinical bone metastasis regional protein expression was assessed with CellTerk Hero-78 and BMPR2 antibodies. Single cell protein expression and spatial analysis was performed in matched patient primary tumors and bone metastases with Akoya Polaris panels.

Results: Myeloid memory was increased in bone metastases and was correlated with BMP signaling. BMP receptor signaling loss recruited macrophages to tumor and reprogrammed macrophages to be pro-inflammatory. BMP signaling loss restricts prostate cancer flank tumor growth and increased overall survival. 

Conclusions: Distinct myeloid metastases exhibited myeloid memory and BMP signaling which could allow for precision immunotherapy treatments to prevent myeloid suppression in mBC.

Bone Morphogenetic Protein Signaling in Myeloid Cells

- BMPs are regulators of differentiation for many cell types.
- BMPs promote bone formation.
- BMPs exhibit context-dependent roles in cancer.
- BMPs impact myeloid phenotypes.
- BMP-2 promotes pro-inflammatory macrophages.
- BMP-4 promotes anti-inflammatory macrophages.
- BMPRIA-LysMCer ckO transgenic mouse model.
- BMP signaling loss restricts bone marrow myeloid progenitors.
- BMP signaling loss restricts prostate cancer flank tumor growth.
- BMP signaling loss recruits macrophages to tumor and reprograms macrophages to be pro-inflammatory.
- For more information see: Ihle, CL et al., Frontiers in Oncology, 2020.

Breast Cancer Bone Metastasis Patient Cohort

Investigating the Tumor Microenvironment in Breast Cancer Patient Bone Metastases, Hematoxylin and eosin staining of a breast cancer patient's bone metastases demonstrated distinct features of lytic bone lesions, tumor cells,stroma, and immune cells. We have a cohort of 47 metastatic bone archival FFPE samples from non-treatment naïve breast cancer patients at the University of Colorado Cancer Center. The majority of samples were collected from primary tumors and ER+/PR+ bone metastases. From this cohort, metastatic breast cancer patient bone metastases were selected which had ER+ bone metastases and lytic bone pathology for analysis of their tumor microenvironment.

Myeloid Gene Expression in Bone Metastases

- Breast Cancer Patient Bone Metastases Exhibit Distinct Myeloid Gene Signature. Gene expression was assessed from RNA isolated from 14 archival FFPE bone biopsies with the NanoString Human Immune Oncology 360 gene expression panel. Distinct separation of bone metastases based on high (n=7) and low (n=7) myeloid gene expression signatures was found. Differential gene expression analysis between high and low myeloid gene expression signatures revealed that 102 myeloid cancer gene signature genes were upregulated in the high myeloid gene signature patients. Low bone metastases were associated with increased expression of cytokines, chemokines and growth factors.

Myeloid Cell Characterization by Digital Spatial Profiling

- Myeloid Gene Expression in Bone Metastases
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Myeloid Signature in Breast Cancer Bone Metastases Impacts Immune Phenotypes & Clinical Outcomes. nCounter gene expression analysis of high vs low myeloid gene signature patients revealed altered immune cell gene signatures in the tumor microenvironment of bone metastases. High myeloid gene signature patients have significantly longer overall survival from the time of bone metastasis diagnosis.

Mouse Models of Myeloid Memory & Breast Cancer

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Multiplexed Immunohistochemistry of Bone Metastases

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Multiplexed Immunohistochemistry Analysis of Macrophage Phenotypes. The Vectra Polaris multiplex immunohistochemistry staining platform was used to analyze the single cell protein expression of 12 breast cancer bone metastasis patient samples. Inform analysis allowed for tissue segmentation, single cell segmentation and cell phenotyping of the tumor microenvironment. Spatial analysis revealed macrophages are enriched in the stroma of bone metastases. Nearest neighbor analysis showed macrophages are closest to tumor cells, myeloid cells, and other macrophages, and farther from neutrophils and T cells.

BMP Modulation of Myeloid Memory in Bone Metastases

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BMP inhibition during myeloid memory training promotes tumor growth by restricting metastasis growth. Mice underwent trained immunity with an intraperitoneal injection of β-glucan 7 days prior to receiving an intratibial injection of syngeneic MMTV-PyMT Bone clone cells. Mice were treated with BMP inhibitor LDN-193189-2HCl or water control using osmotic pumps. Mice with 28-day delivery pumps with LDN-193189-2HCl implanted 7 days after intratibial bone metastasis formation did not alter bone metastasis growth. Mice with 7-day delivery somatotropins with LDN-193189-2HCl implanted during β-glucan training had increased bone metastasis growth, but decreased secondary metastasis to the lungs.

Summary & Next Steps

Summary:
- Bone metastasis patients with inflammatory myeloid genes have longer overall survival.
- Bone metastases are enriched for inflammatory myeloid genes & BMP signaling genes compared to primary tumors.
- BMP inhibition during myeloid memory training promotes metastasis growth but limits secondary metastasis.
- BMDM have decreased BMP signaling after LPS stimulation & increased IL-6 gene expression after LPS stimulation & BMP inhibition.

Ongoing Experiments:
- Polyclonal antibody 41AA Polaris signaling antibody panel.
- In Vitro: Trained BMDM conditioned media & co-cultures with breast cancer spheres.

Acknowledgements

We would like to thank Sabrina Wright-Hobart for her collaboration and metastatic breast cancer patient advocacy. We thank Ryan Ottery, Chey Yen,嫚, Shih Lang, Yan Liang, Wang Li, and Doug Hanel for optimizing panels and executing multiplexed immunohistochemistry analysis. We would like to thank Sabrina Wright-Hobart for her collaboration and metastatic breast cancer patient advocacy. We thank Ryan Ottery, Chey Yen,嫚, Shih Lang, Yan Liang, Wang Li, and Doug Hanel for optimizing panels and executing multiplexed immunohistochemistry analysis. We would like to thank the University of Colorado Cancer Center Tissue Biobanking and Histology Shared Resource (P30CA046592) and the U.S. National Institutes of Health through the National Cancer Institute (P30CA046592) for support.

Biorender, the Vectra P® Imaging System, Sigma-Aldrich, the NanoString nCounter® phenotype panel, and conditioned media.

Bone Marrow Derived Macrophage Treatment with Trained Immunity & BMP Inhibitor. Mice underwent trained immunity with an intraperitoneal injection of β-glucan 7 days prior to collecting bone marrow and generating bone marrow derived macrophages (BMDM). 8 days after plating the BMDM, cells were treated with LDN-193189-2HCl or water control. On day 7 the cells were stimulated with LPS for 24hrs prior to collecting RNA and conditioned media. ID1 and IL-6 gene expression was analyzed by RT-qPCR.