The Metastatic Breast Cancer Microenvironment in Bone Exhibits Unique BMP Signaling

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Abstract

(Breast cancer (BC) patient prognosis has improved for localized BC, yet metastatic BC continues to cause high mortality with a 5 year survival rate of only 27%. Approximately 70% of BC metastases occur in the bone, and tumor progression and enhanced macrophage infiltration are driven by myeloid gene signature enriched patient bone samples. Myeloid memory is modulated by BMP signaling.

Investigating the Tumor Microenvironment in Breast Cancer Patient Bone Metastases. A cohort of 47 breast cancer bone metastasis samples from metastasis naïve breast cancer patients at the University of Colorado Cancer Center were generated, with the majority of cases exhibiting ER+ primary tumors and ER+ bone metastases. Analysis of complete blood counts after 6 weeks of metastatic tumor growth revealed a 5 year survival rate of only 27%. Approximately 70% of BC metastases occur in the bone, with a tumor microenvironment (TME) composed primarily of BC cells, immune cells, and bone cells. In these cellular compartments, bone morphogenetic protein (BMP) expression exhibits unique TME dependent tumor promoter and suppressor effects. Previous studies in our lab have found BMPs promote myeloid progenitors, polarization of M2 macrophages, and tumor progression in a BMPR1α/LysMCre conditional knockout mouse model. Yet to establish BMPs as a viable target for the treatment of metastatic breast cancer, the mechanisms behind BMPs promoting BC bone metastasis must be investigated. To further investigate whether BMP-dependent myeloid heterogeneity in cancer; we have utilized a cohort of non-treatment naïve BC patient bone biopsies to unveil the distinct myeloid populations in the TME of BC bone metastases. Differential gene expression analysis revealed a subset of patient samples with a high myeloid gene signature, corresponding with increased chemokine, cytokine and JAK/STAT signaling gene pathways in addition to enhanced BMP signaling. Digital Spatial Profiling via the NanoString GeoMx platform and multiplex immunohistochemistry staining via the AKOYA Vectra Polaris platform were used to analyze the spatial context of the TME in our cohort of patient bone. We found enhanced BMP signaling, and myeloid heterogeneity and BMP2 signaling in the TME. Tumor progression and enhanced macromolecular in the TME. This precision oncology analysis of the unique landscape of the metastatic bone TME revealed BMP driven phenotypes in distinct TME immune compartments. To then determine if the TME features observed in the metastatic bone samples was reflective of altered innate trained immunity regulated by BMPs, we investigated the requirement for BMP signaling in myeloid inflammatory responses both in vitro and in a mouse model of BC bone metastases. We found BMPs ability to modulate macrophages to undergo innate trained immunity functions and BC bone metastases alter myeloid inflammatory responses in mice. Investigating the heterogeneity and functions of myeloid cells in the TME of bone metastatic BC will help advance therapeutic target development for BC patients with bone lesions. Expanding therapeutic options for metastatic BC patients will improve patient quality of life and reduce deaths caused by metastasis.

Macrophage Characterization by Digital Spatial Profiling

Digital Spatial Profiling of Breast Cancer Bone Metastases Reveals Macrophage Phenotypes. NanoString Digital Spatial Profiling (DSP) was used to determine the distinct phenotypic characteristics of the TME macrophages, and T cell regions of tumor induced bone disease from metastatic patients. Regions of interest (ROIs) were selected in 7 patient biopsies, with 4 tumor, macrophage, and T cell ROIs selected for each patient. Protein expression was analyzed by antibody counts in ROIs for each antigen.

Myeloid Memory in BMPR1α−/− Macrophages

Loss of BMP Signaling Alters Myeloid Memory. Bone marrow macrophage cell lines derived from wild type and LysMCre BMPR1α−/− mice underwent innate immune entrainment. Cells were treated with Beta-glucon for 24hrs followed by 5 days of culturing when LPS for 24hrs followed by RNA collection. Gene expression analysis measured BMP signaling through ID1 and inflammatory response through IL-6 and TNFa.

Modulated BMP Signaling in Myeloid Memory

Macrophage Treatment with BMP-2 or BMP Inhibitor Alters Myeloid Memory. Rna-Seq 4.7 macropages were treated with BMP-2 or BMP inhibitor LDN-193189 24hrs before undergoing innate immune entrainment, for a total of 48hrs of treatment with BMP stimulus or inhibitor. Cells were subsequently treated with Beta-glucon for 24hrs followed by 6 days of culturing when LPS for 24hrs followed by RNA collection. Gene expression analysis measured BMP signaling through ID1 and inflammatory response through IL-6 and TNFa.

Trained Immunity in Mouse Bone Metastases

Preliminary Evidence of Trained Immunity Modeled in Mouse Breast Cancer Bone Metastases. To investigate how trained immunity functions in mice with metastatic bone lesions, F3B mice were treated with Beta-glucon 7 days prior to receiving LPS to mimic macrophage activation of M1-like M2-like polarization. 24hrs before undergoing innate immune entrainment, for a total of 48hrs of treatment with BMP stimulus or inhibitor. Cells were subsequently treated with Beta-glucon for 24hrs followed by 6 days of culturing when LPS for 24hrs followed by RNA collection. Gene expression analysis measured BMP signaling through ID1 and inflammatory response through IL-6 and TNFa.

Summary & Next Steps

- Myeloid gene signature enriched patient bone samples have enhanced inflammatory signaling (JAK/STAT & chemokine/cytokine), myeloid infiltration, M2-like polarization, & BMP signaling
- Myeloid memory is modulated by BMP signaling in vivo
  - Trained immunity increases ID1 gene expression
  - LDN-193189 treatment increases macrophage inflammatory response
- Trained immunity can be modeled in metastatic bone metastases
- Next Step: Investigate if BMP signaling is required for trained immunity in a mouse model of breast cancer bone metastases

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