Presence of Metastatic Breast Cancer Induces Gene Expression Changes at the Fracture Site

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INTRODUCTION:

Pathological fractures secondary to metastatic breast cancer demonstrates impaired fracture repair and nonunion (**Figure 1**). Outside of antiresorptive agents, there have been few advances in targeting cancer-disrupted fracture healing. A thorough understanding of the gene expression changes induced by the presence of metastatic breast cancer is critical to addressing poor fracture healing. We apply spatial transcriptomics showing that the presence of metastatic breast cancer cells in the fracture microenvironment induces inflammatory changes not seen in normal fracture repair.

METHODS:

Breast cancer cells (MDA231, MCF7, HC1806, mammary cell clones, matrigel, 4T1) were inoculated via intramedullary injection ($1x10^4$) following femoral osteotomy in female nude or immune competent BALB/c mice (age 16-20 weeks; n=4-6/group). Mice were imaged with microCT and green-fluorescence protein imaging and sacrificed at Day 4, Day 7, Day 14, and Day 28. Representative tissue sections were processed with Visium (10x Genomics) spatial transcriptomics. Spatial transcriptomics uses localized probes to identify gene expression corresponding to histology. Data was filtered and normalized with SCTransform. Graph-based clustering was performed, and spatial expression of inflammatory cytokines, chemokines, growth factors, osteoclastogenic, and anti-osteoclastogenic data was analyzed with pathway analysis and StringDB clustering.

RESULTS:

Radiographic and histological imaging of the pathological fracture samples demonstrated non-union and impaired callus formation compared to normal controls (**Figure 2**). Normal fracture samples at Day 4, 7, 14, and 28 demonstrated appearance of fracture-specific cells at the fracture line, callus formation, and remodeling with identifiable clusters of gene groups (**Figure 3**). The MDA231 and 4T1 inoculated samples demonstrated a hypoplastic fracture callus and nonunion. For instance, MDA231 inoculated samples showed downregulation of key repair genes such as *Tnc, Bglap,* and *SPARC* ($p<0.05$, false-discovery rate <0.01). We also identify the bridging interzone between adjacent soft calluses as a critical region of fracture repair, with downregulation of 78 signaling genes compared to normal fracture samples ($p<0.05$, false-discovery rate <0.01). In the 4T1, we find minimal callus formation but heavy osteoclastic, inflammatory, and invasive gene expression (*Spp1, Fut8, Emp1, Clic1*). Overall, pathway analysis shows alteration in collagen, protein processing, cellular signaling, and translation regulation (Protein–Protein Interaction Enrichment p-value < 0.0001 for network analysis).

DISCUSSION AND CONCLUSION: The presence of breast cancer cells derails fracture-healing cells, invokes deranged signaling pathways, and induces a highly inflammatory microenvironment that likely contributes to impaired fracture repair. Additionally, there is evidence of disrupted temporal patterning of fracture repair and induction of an inflammatory milieu leading to impaired callus formation and non-union.

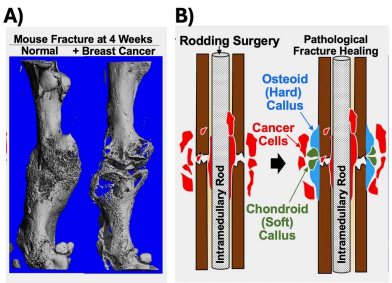

Figure 1. A) Presence of breast cancer cells leads to poor callus formation as shown on microCT. **B)** Fracture healing undergoes formation of soft callus and hard callus.

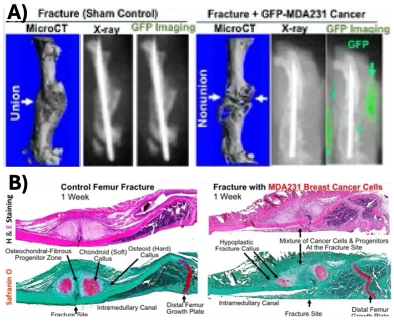

Figure 2. A) Cancer cells lead to non-union. **B)** Cancer cells lead to impaired callus formation.

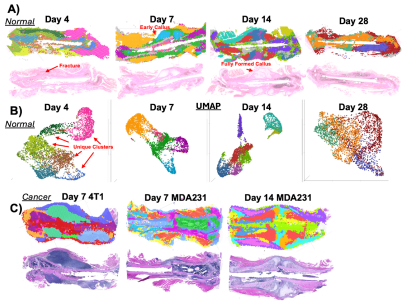

Figure 3. A) Normal fracture callus formation (Day 4, 7, 14 and 28). **B)** UMAP gene clustering. **C)** Fractures with presence of 4T1 or MDA231.