Zinc Finger MYND-Type Containing 8 (ZMYND8) Promotes Bone Metastasis Progression in Breast Cancer

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BACKGROUND

Breast cancer bone metastasis remains a major clinical challenge, with a five-year survival rate of only approximately 22% following distant recurrence. Treatment is hindered by therapeutic resistance, frequent metastasis relapse, and the emergence of secondary metastases.

ZMYND8, a chromatin reader, has emerged as a key player in cancer biology. It is involved in histone modification recognition, DNA repair, and can also promote angiogenesis under hypoxic conditions (**Fig. 1A**). It is also a prognostic marker in breast cancer (**Fig. 2B**). Our preliminary data show that ZMYND8 is significantly upregulated in bone metastases compared to naïve tumor cells in an MCF7 estrogen receptor-positive (ER+) breast cancer model (**Fig. 2A**). This suggests that ZMYND8 may play an active role in metastatic progression.

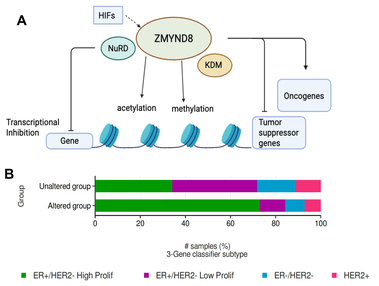


Figure 1. ZMYND8 mechanism and alteration in breast cancer subtypes. A- ZMYND8 as an epigenetic regulator. **B-** ZMYND8 associates with worse survival outcome in breast cancer. **C-** ZMYND8 alterations are enriched in highly proliferative HR+/Her2- breast cancer.

METHODS

In a multiorgan metastasis model, we performed dual injection to the bones (intra-iliac injection) and the mammary glands (mammary fat pad injection). Tumor cells were derived from organs that had metastases, sorted and analyzed with single cell RNA sequencing.

We analyzed proteomic data from TCGA BRCA patients and performed Gene Set Enrichment Analysis (GSEA) and Gene Ontology (Biological Process) analysis to identify pathways enriched for ZMYND8.

Immunofluorescence staining was performed on patient samples with ZMYND8 antibody. Each patient has a primary tumor sample and a bone metastasis sample. 5 patient paired samples were analyzed.

MCF7 cells were cultured alone or co-cultured with osteoblasts (FOB), and ZMYND8 expression was quantified with immunofluorescent staining.

DNA damage was studied with γH2AX immunofluorescent staining. Effect of estrogen inhibition was studied with ovariectomy and aromatase inhibitor in a ZR75-1 mouse model.

We developed ZMYND8 knockdown in MCF7 cell lines expressing firefly luciferase. Cell proliferation was evaluated by BrdU assay. Wound healing assay was performed for 96H after starving the cells for 24h to reduce cell growth. Wound closure was assessed at 96h post scratching. Colony formation assay was performed at 14 days.

ZMYND8 knockdown and control MCF7 cells were delivered to bone via intra-iliac artery injection to NU/J mice and tumor growth was measured with IVIS.

RESULTS

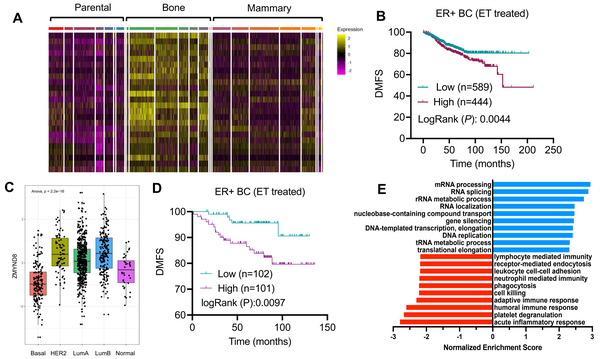


Figure 2. Clinical relevance of ZMYND8 in ER+ breast cancer. A- ZMYND8 is upregulated in the bones in a multi-organ metastasis MCF7 xenograft mouse model. Heatmap represents the top differentially expressed genes in all cell clusters identified from parental MCF7 cells, bone, and mammary gland and based on single-cell RNA-seq. ZMYND8 is the second most differentially expressed gene. **B-** ZMYND8 is a prognostic marker for endocrine treated distant metastasis free survival. **C-** ZMYND8 is highly expressed in luminal A/B and HER2 subtypes. **D-** Top 50 genes positively associated with ZMYND8 in TCGA BRCA breast invasive carcinoma correlate with worse survival. **E-** Top upregulated and downregulated pathways correlated to ZMYND8 protein expression.

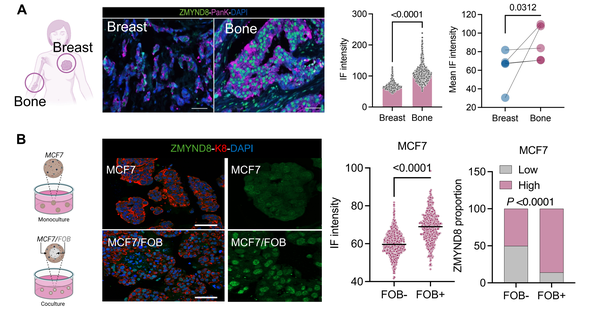


Figure 3. ZMYND8 is enriched in the bones. Immunofluorescent staining of ZMYND8 (green), Keratin 8 (red), Pan Keratin (magenta), DAPI (blue) **A-** ZMYND8 is more highly expressed in bone metastasis than primary breast tumor. **B-** ZMYND8 is more highly expressed in coculture of MCF7 and FOB than monoculture of MCF7.

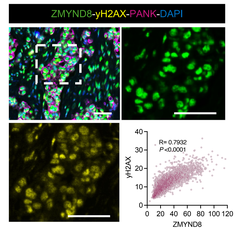


Figure 4. ZMYND8 co-localized with DNA damage sites. Immunofluorescent staining of γH2AX indicated a linear correlation between ZMYND8 expression and DNA damage.

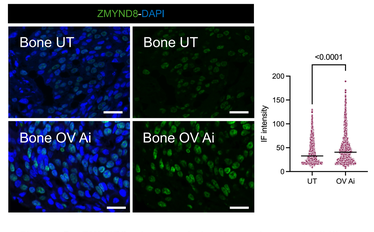


Figure 5. ZMYND8 is elevated after estrogen inhibition. Immunofluorescent staining shows higher ZMYND8 in mice bearing ZR75-1 (ER+) bone metastasis that underwent ovariectomy and aromatase inhibition than untreated.

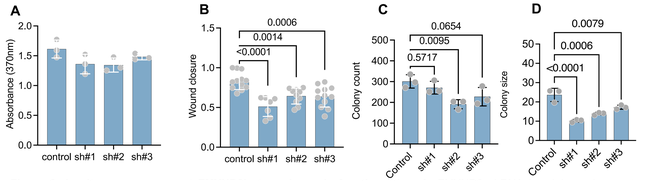


Figure 6. *In vitro* assays suggest ZMYND8's tumorigenesis functionalities. A- ZMYND8 shRNA knockdown does not change MCF7 proliferation. **B-** ZMYND8 knockdown decreases wound healing ability of MCF7 cells at 96H. **C-D-** ZMYND8 knockdown decreases colony formation ability at 14 days.

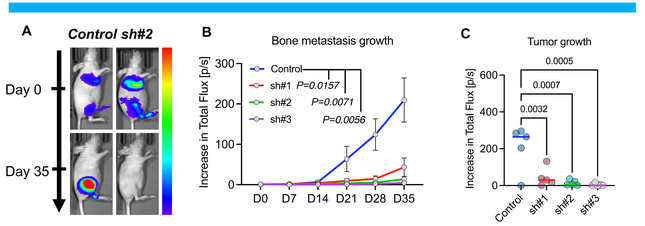


Figure 7. MCF7 cells with shRNA knockdown of ZMYND8 impeded bone metastasis growth *in vivo*. NU/J mice were injected with 500,000 MCF7 cells in the intra-iliac artery of the right leg at Day 0, and tumor growth was monitored by IVIS for up to 35 days. **A-** Representative pictures of bioluminescence of tumor growth at Day 0 and Day 35 of control and s hRNA knockdown MCF7 cells *in vivo*. **C-** Growth curve of control and shRNA knockdown tumor cells in mice. **D-** At Day 35, mice in ZMYND8 knockdown groups had significantly less tumor burden than mice in the control group.

CONCLUSIONS

Our results suggest that ER+ breast cancer patients with higher expression of ZMYND8 have worse survival outcomes compared to patients with low ZMYND8. Moreover, ZMYND8 is more highly expressed in the bones compared to other metastatic organs or primary sites of breast cancer, indicated both on mRNA and protein level, suggesting a bone-specific effect on the increased expression.

ZMYND8 is associated with enrichment of pathways linked to chromatin organization, transcriptional regulation, and other nuclear regulatory processes, consistent with ZMYND8's role as an epigenetic reader. In contrast, pathways negatively enriched are predominantly involved in immune-related and stress-response biological processes, suggesting that reduced ZMYND8 expression may shift tumors toward a more inflammatory and immune-active state.

In vitro and *in vivo* studies show that ZMYND8 is associated with tumor initiation, migration, and tumor growth in the bones. We also provided evidence for the possible implication of ZMYND8 in DNA damage repair mechanism as well as endocrine therapy resistance of bone metastases. Collectively, these results suggest ZMYND8 as a critical regulator of breast cancer bone metastatic progression and highlight its potential as a therapeutic target in ER+ breast cancer.

Ongoing and future research includes *in vivo* studies to understand ZMYND8's role in bone metastasis progression, mechanistic studies to investigate its molecular activities, and its therapeutic implications. We will conduct studies to uncover novel epigenetic regulation pathways of ZMYND8 in the context of bone metastasis driven by ER+ breast cancers.

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