FGFR1 promotes brain metastases of ER+ breast cancer through canonical and non-canonical mechanisms in young and aged hosts

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Background

- Estrogen receptor positive (ER+) breast cancer is the most common subtype of breast cancer (BC).
- Despite showing the lowest incidence of brain metastasis (BM) among BC subtypes, ER+ BCBMs still account for a significant fraction of BCBM (Figure 1A).
- ER+ BCBMs occur most frequently in older and post-menopausal women, following years of estrogen-suppression therapies (Figure 1B,C).
- *The mechanisms underlying brain metastatic colonization in ER+ BC and the extent to which aging and E2-deprivation contribute to brain metastases remains unknown.*

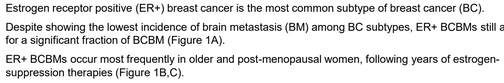
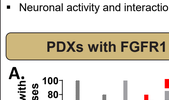


Figure 1. Subtype and age characteristics of a cohort of 85 BCBMs at UCAMC. **A.** 48% of brain metastases are from patients with ER+ primary tumors. Most ER+ BM patients are >50 years old **(B)** and/or post-menopausal (**C**)

TME-mediated activation of FGFR1 drives ER+ BC primary tumor and metastasis outgrowth.

- FGFR1 amplification is the only genetic change that predicts risk of late recurrence in early ER+ BC treated with aromatase inhibitors.
- FGFR1 aberrations are a risk factor for BCBM development in all subtypes and predict poor prognosis.
- Paracrine activation of FGFR1 in ER+ BC has been shown to promote primary tumor progression in post-menopausal and obese BC mouse xenograft models, and in bone metastatic colonization.
- ER+ BC PDX with FGFR1 amplification showed increased ability to colonize the brain following experimental dissemination of cancer cells (Figure 2).
- FGF2/FGFR1 signaling plays key roles in brain function and decreases with E2 deprivation and aging.
- FGFR1 also binds NCAM1, which is expressed on the surface of neurons and glial cells, present at much higher concentrations in the brain than FGFs, and known to activate FGFR1 signaling.
- FGFR1 regulates neural progenitor growth, synaptogenesis, self-renewal of adult neural stem cells, and delivery/insertion of glutamate receptors into postsynaptic membrane.
- Neuronal activity and interactions with neurons can drive cancer growth in the brain for glioblastoma and BM.

PDXs with FGFR1 amplification colonize the brain at high frequencies



B.

PDX	UCD4 6	UCD4	UCD12	UCD6 5
% with BM	0%	20%	40%	80%
FGFR Copy #	No amp	5x FGFR 3	12x FGFR 1	14x FGFR 1
FGFR1 mRNA	13.0	15.7	30.7	339.5

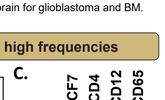


Figure 2. **A.** Metastasis frequencies of ER+ PDX upon intracardiac injection in young+ E2 mice. **B.** PDX FGFR copy number and mRNA expression (FPKM). **C.** FGFR1 and pFGFR1 expression in ER+ BC lines.

HYPOTHESIS:

Canonical and non-canonical activation of FGFR1 by astrocytes and neurons promotes FGFR1-dependent ER+ BCBM in E2-high/young and E2-low/aged hosts.

ER+ BCBM PDX can colonize the brain of older/E2-low female mice

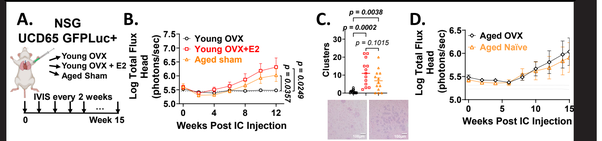


Figure 3. **A.** UCD65 cells were injected intracardially into young OVX NSG mice ± E2 or in aged (52+ weeks) sham mice. **B.** IVIS signal of the head over time. **C.** Histological quantification of BM clusters (H&E). **D.** UCD65 cells were IC injected into aged OVX or naive NSG mice. Head IVIS over time. Gray lines: IVIS signal from tumor-free mice.

FGFR1 drives growth of ER+ BC in organotypic coculture and *in vivo*

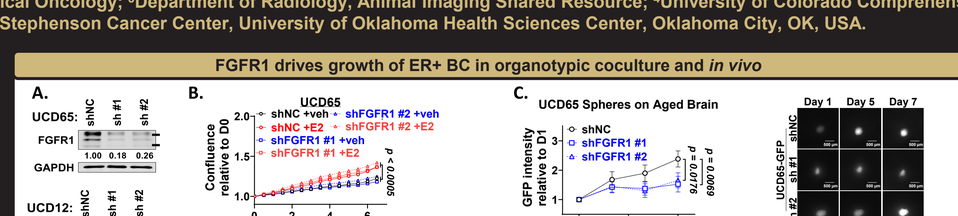
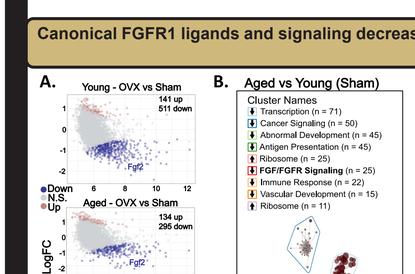
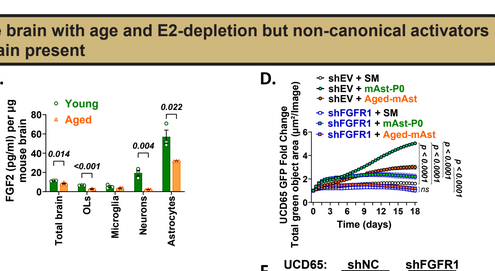


Figure 4. **A.** FGFR1 expression in shNC and shFGFR1 cells. Bars: 100kDa and 150kDa. **B.** Growth of shNC and shFGFR1 UCD65 cells treated with vehicle (EtOH) or 10nM E2. Live cell imaging confluence normalized to time 0. **C.** Fold change in GFP+ intensity over time for UCD65 spheroid growth on aged organotypic brain slices. **D.** Head metastatic progression of shNC and shFGFR1 UCD65 GFPLuc+ cells injected intracardially into (**D**) young OVX+E2 mice and (**E**) aged naïve NSG mice.

Canonical FGFR1 ligands and signaling decrease in the brain with age and E2-depletion but non-canonical activators remain present





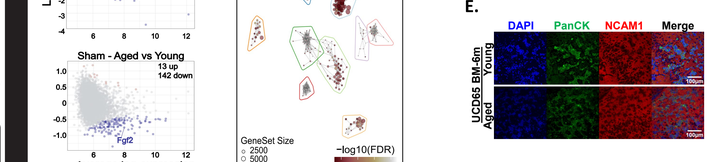


Figure 5. **A.** GeoMX DSP Nanostring analysis of late stage UCD65 BM in OVX or sham, aged and young NSG mice. Volcano plots of indicated pairwise comparisons of differentially expressed genes in GFAP+/Iba1+ glial cells surrounding BMs. Differentially upregulated (red) and downregulated (blue) genes are shown. **B.** Top 9 differentially expressed clusters of pathways (significant GSEA signatures sharing >35% overlapping genes) for aged versus young sham glial ROIs. **C.** FGF2 ELISA from brain isolated cells from young and aged female mice. **D.** Fold change growth of UCD65 shEV and shFGFR1 cells alone or with mAst-P0 or Aged-mAst in E2-free media. **E.** PanCK and NCAM1 IF staining in UCD65 BCBM in young and aged NSG mice. **F.** Immunoblot from UCD65 shNC and shFGFR1 cells treated with 10ng/mL FGF2, 250nM NCAM1, or vehicle. **G.** Pretreatment of UCD65 cells with BGJ398 or vehicle prior to treatment as in F. **H.** NCAM1 expression in SH-SY5Y cells expressing shEV or shNCAM1. **I.** pFGFR1 staining in UCD65-GFP+ cells co-cultured with shEV and shNCAM1 SH-SY5Y cells. pFGFR1 quantification in UCD65, UCD12, MCF7-FGFR1-OE and MCF7-EV cells cultured on top of shEV or shNCAM1 SH-SY5Y cells.

NCAM1 promotes neural interactions and migration of ER+ BC cells

Figure 6. **A.** Fold change of UCD65 cells treated with vehicle, 10nM E2, 10ng/mL FGF2, or 250nM NCAM1 for two weeks. **B.** Log(FDR) of significant neural gene signatures differentially enriched or repressed following NCAM1 treatment. **C.** Time lapse images of GFP+ shNC and shFGFR1 UCD65 cells plated on coverslips with primary hippocampal neurons. Graph shows mean distance per cell within each image. **D.** UCD12 and UCD65 cells were plated on a monolayer of shEV or shNCAM1 SY5Y cells as in C. Graph shows mean distance per cell.

FGFR1 kinase inhibition is only effective to block early BCBM colonization

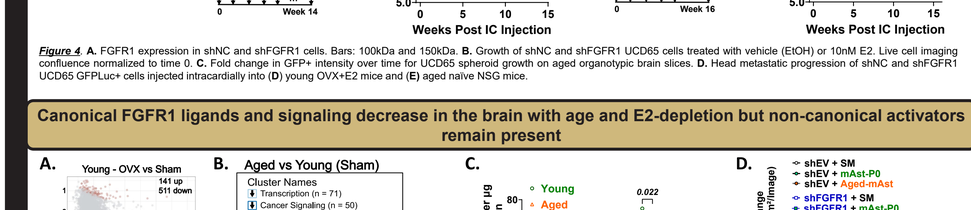
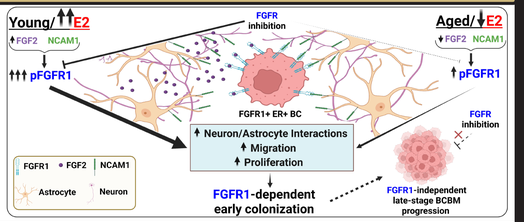


Figure 7. **A.** PanCK and pFGFR1 in late-stage UCD65 BCBMs in young and aged mice. **B.** pFGFR1 quantification in UCD65 cells 5 days after intracardiac injection in young and aged mice. **C.** UCD65 GFPLuc+ cells were IC injected into OVX-E2 young mice receiving vehicle (1% CMC) or BGJ398 (30mg/kg) 5x/week PO starting at the time of injection (W0-12) or 7 weeks post-injection (W7-12). Graphs is head metastatic burden over time. **D.** Ex *vivo* brain metastases at endpoint. **E.** UCD65 GFPLuc+ cells were IC injected into aged mice and treated with vehicle or BGJ398 starting at the time of injection (W0-14,) or 10 weeks post-injection (W10-14). **F.** pFGFR1 quantification in UCD65 cells 5 days after IC injection in naïve mice treated with vehicle or BGJ398 1 day post- or 5 days pre-injection.

Conclusions

- FGFR1 promotes ER+ BC cell growth and migration through TME-mediated activation.
- Canonical FGF2 ligand expression and signaling decreases in the brain with age and E2-depletion.
- TME-mediated FGFR1 activation is critical for early but not late stages of ER+ BCBM colonization.



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