FGF1 REGULATES BREAST CANCER GROWTH AND METABOLIC REPROGRAMMING THROUGH ETV4

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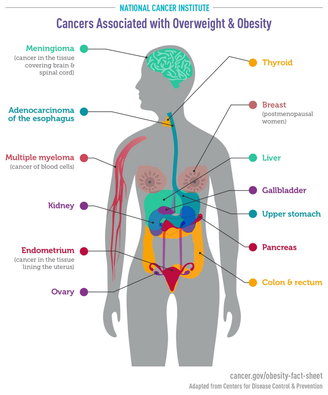
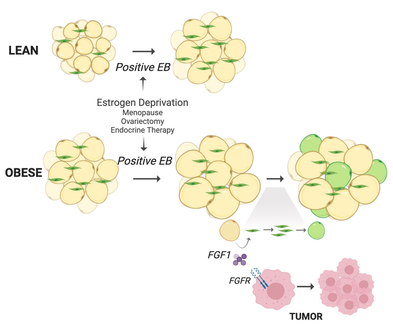
BACKGROUND

- Obesity is associated with resistance to breast cancer endocrine therapies.
- Adult weight gain in women with obesity is an independent prognostic factor for breast cancer.
- Weight gain supports fibroblast growth factor (FGF1) production by adipose tissue and promotes tumor growth.
- FGF1 stimulated Estrogen receptor (ER) phosphorylation and breast cancer cell glycolysis.
- ETS variant 4 (ETV4) is induced by FGF1 in many ER+ breast cancer cells.
- ETV4 alters ER DNA binding activity and regulates glycolytic gene expression in cancer cells





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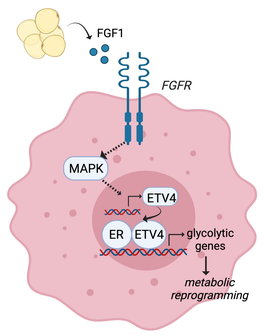
HYPOTHESIS

FGF1 promotes breast tumor growth through ETV4 and glycolytic metabolism



METHODS

- ER+ MCF7 tamoxifen resistant (TAMR) or MCF7 Parental (MCF7p) cells were used for these studies
- ETV4 knockdown cells were made with stable shRNA lentiviral transduction
- ETV4 over-expressing cells were made with lentiviral transduction
- Incucyte Live-Cell imaging was used to measure cell proliferation
- Immunoblot was used to evaluate glycolytic enzyme levels
- Quantitative PCR was used to measure the expression of metabolic genes in cells with and without ETV4
- Seahorse metabolic flux analysis was used to quantify cellular oxygen consumption and acidification rates (OCR and ECAR).
- Microarray was used to measure the gene expression profile in ETV4 overexpressing and control MCF7TAMR and MCF7p cells

RESULTS

ETV4 associates with obesity, FGF1, & breast cancer prognosis

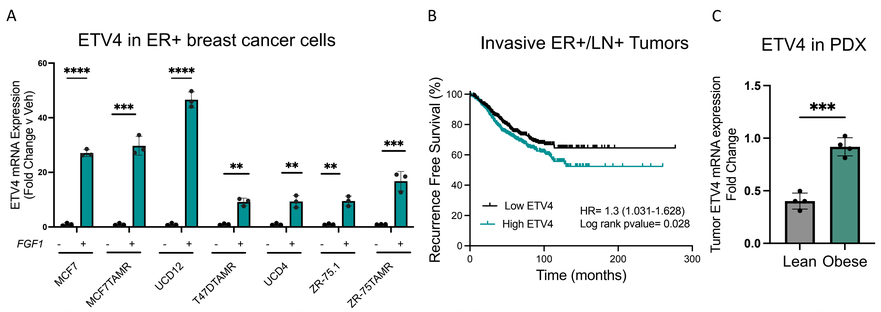


Fig 1. ETV4 in human breast cancer cells. (A) ETV4 expression by qPCR in human breast cancer cells treated with vehicle or FGF1 for 24 hours; data expressed as fold change FGF1 vs vehicle in each cell line. (B)) Kaplan-Meier plot showing recurrence-free survival of all patients with ER+, lymph node+ tumors associated with ETV4 expression. N=1065 cases. (C) ETV4 gene expression in human ER+ PDX tumors from obese and lean mice.

ETV4 mediates FGF1-induced cell proliferation

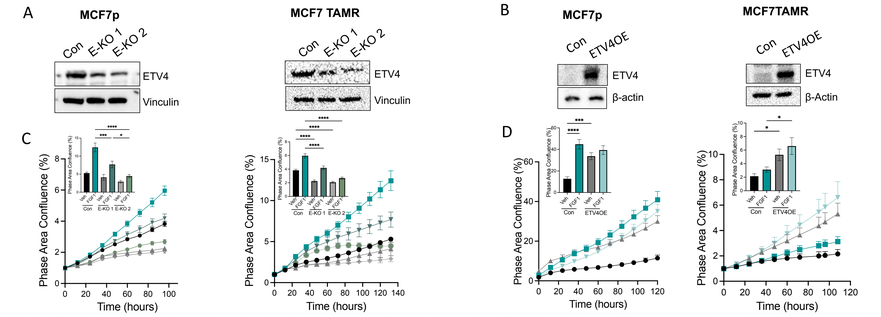


Fig 2. ETV4 regulates cell proliferation (A) Immunoblot of ETV4 in Control or ETV4 knockdown (ETV4KO) MCF7p and MCF7TAMR cells. (B) Immunoblot of ETV4 in Control or ETV4 overexpressing MCF7p or MCF7TAMR cells. (C) Cell proliferation measured by Incucyte Live-Cell Imaging in Control and ETV4KO cells treated with vehicle of FGF1. (D) Cell proliferation in Control and ETV4 overexpressing cells treated with vehicle of FGF1 for 72 hours. (inset) Statistical analysis of cell viability in the cells 72 hours after treatment with vehicle or FGF1. Insets in C-D cell viability at the final timepoint of proliferation, t-test.

ETV4 is necessary for FGF1 induction of glycolysis

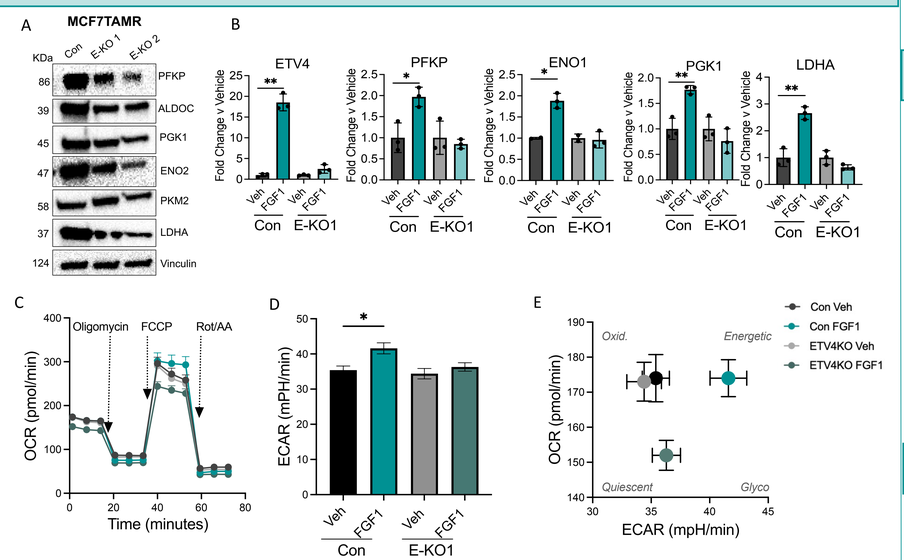


Fig 3. Regulation of glycolysis by ETV4 (A) Immunoblots of PFKP, ALDOC, PGK1, ENO2, PKM2, LDHA, in Control and ETV4KO MCF7TAMR cells. (B) Expression of glycolytic genes measured by qPCR in Control and ETV4KO MCF7TAMR cells treated with vehicle and FGF1 for 24 hours. (C) Kinetic graph of seahorse Mito stress test showing oxygen consumption rate (OCR) (D) Extracellular acidification rates (ECAR) from the assay in (c). (E) Seahorse energy map showing OCR and ECAR in Control and ETV4-KO MCF7 TAMR cells treated with vehicle or FGF1 for 24 hours

ETV4 augments glycolysis and alters gene expression

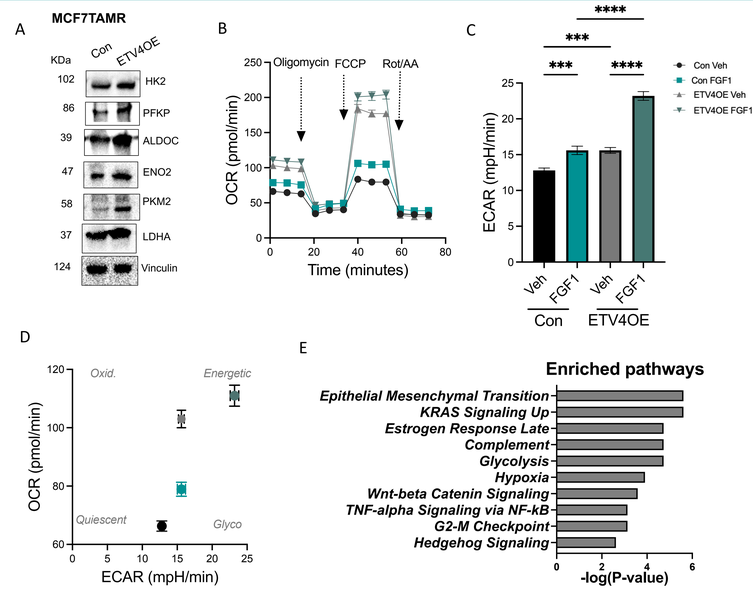


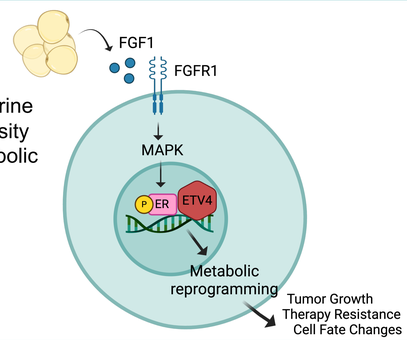
Fig 4. ETV4 promotes glycolysis and aggressive cancer cell gene expression. (A) Immunoblots of HK2, PFKP, ALDOC, ENO2, PKM2, LDHA, in Control and ETV4 overexpressing MCF7TAMR cells. (B) Kinetic graph of Seahorse Mito stress test showing oxygen consumption rate (OCR) (C) Extracellular acidification rates (ECAR) from assay in (b). (D) Seahorse energy map showing OCR and ECAR in Control and ETV4 overexpressing MCF7TAMR cells treated with vehicle or FGF1 for 24 hours (E) Pathway analysis of genes upregulated in ETV4OE MCF7TAMR cells versus control cells. GSEA Molecular Signatures Database Hallmark gene sets are shown.

CONCLUSION

- FGF1 induces ETV4 expression in ER+ breast cancer cells
- High ETV4 expression associates with shorter recurrence-free survival with invasive ER+ tumors and with diet-induced obesity in mice
- FGF1 induces proliferation in breast cancer cells in part through ETV4
- ETV4 regulates glycolytic gene and protein levels in MCF7 TAMR cells
- ETV4 is required for FGF1-induced glucose metabolism in breast cancer cells
- ETV4 regulates expression of genes associated with aggressive breast cancer

Working Model

FGF1 may support breast cancer endocrine therapy resistance in the context of obesity through ETV4 induction and tumor metabolic reprogramming



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