Transcriptional Plasticity in Early Evolving Endocrine Therapy Resistance

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Background - Endocrine Therapy Resistance

- 70% of breast cancers express estrogen receptor (ERα), which is coded by the *ESR1* gene.
- **Tumor cell populations in ER-positive (ER+) tumors are diverse** and may show variable expression of ERα.
- **Endocrine therapy (ET) forms the backbone of treatment of ER-positive (ER+) breast cancers**. These include aromatase inhibitors, tamoxifen, and selective estrogen receptor degraders, used as monotherapy and in conjunction with adjunct targeted therapies.
- Research on mechanisms of ET resistance tends to focus on pathways that cooperate with high ERα activity.
- Given the cellular diversity of ER+ tumors, whether **ET resistance mechanisms can arise from cell populations with lower ER expression** has not been well explored.

Methodology

- Patient-derived xenograft (PDX) mouse models have been developed from human ER+/HER2-negative breast cancer tumors.
- Mice from PDX models of ER+/HER2- breast cancer were treated with endocrine therapy ("**Tam**" – tamoxifen 50 mg/kg PO daily) vs. placebo ("**Control**") for 6 weeks.
- Single-cell RNA sequencing (scRNAseq) and chromatin accessibility analysis with single-cell Assay for Transposase-Accessible Chromatin with sequencing (scATAC-seq) was performed on harvested tumors at 6 weeks.
- Single-cell data was assigned to Seuratv5 objects (*Hao et al., Nat Biotechnol. 2023*), with gene counting and normalization performed with SCTransform, followed by Leiden clustering to define UMAP cell clusters.
- Metacell kinase and transcription factor signature module scores were calculated with UCell based on signatures defined from ARCHS[4] (*Lachmann et al., Nat Commun. 2018*).
- Pseudotime trajectory analysis was performed using Monocle 3 (*Trapnell et al., Nat Biotechnol. 2014).*
- For cell clusters, gene set enrichment analysis (GSEA) was performed with respect to ARCHS[4] kinase and transcription factor (tf) signatures.

Kinase activity correlation with ESR1 expression

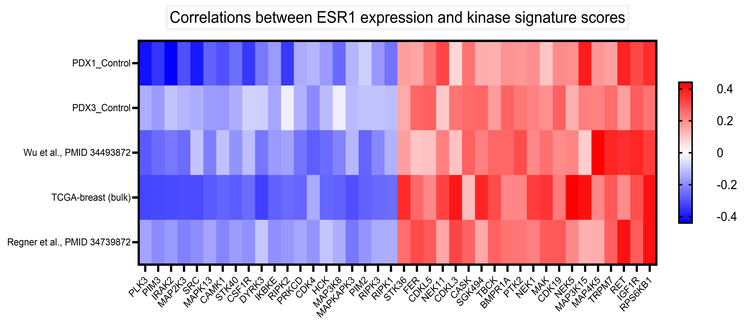


Figure 1: Correlation between *ESR1* expression and kinase activity. Kinase signatures were calculated on scRNAseq metacells from untreated PDX models (PDX1, PDX3) and from published human breast cancer single-cell datasets (Wu et al., Nat Genet. 2021; Regner et al., Mol Cell. 2021), as well as from bulk RNAseq from TCGA-breast (Koboldt et al., Nature 2012). Consistently across PDX models and datasets, kinase signatures for *PLK3, PIM3, and IRAK2* notably demonstrated negative correlation with *ESR1* expression. Conversely, kinase signatures for *IGFR1 and RET* positively correlated with *ESR1* expression.

Single cell pseudotime trajectory analysis of the PDX1 xenograft model – Tamoxifen vs. Control

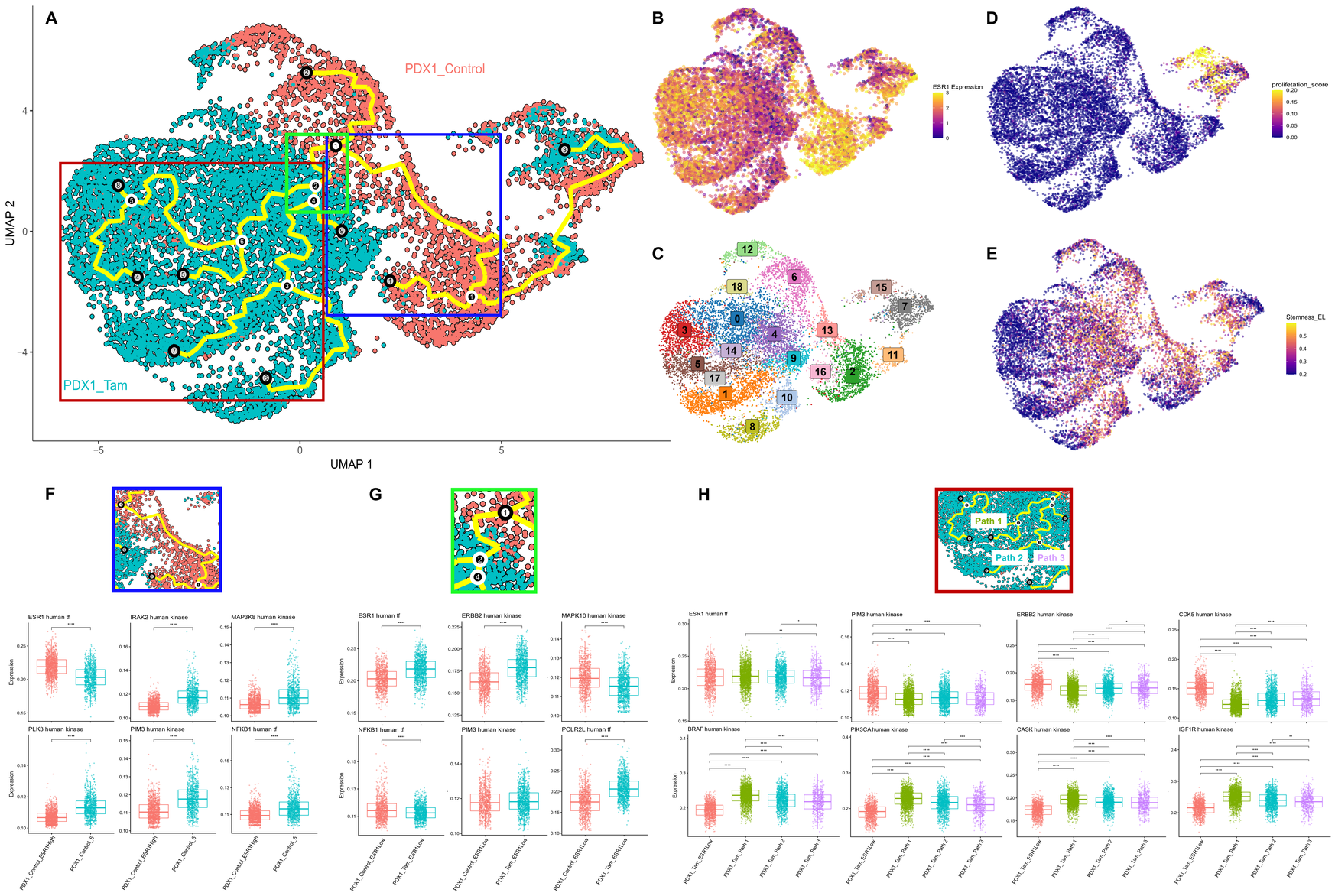


Figure 2: scRNAseq and trajectory analysis of PDX1 tumors treated with tamoxifen (PDX1_Tam) vs. placebo (PDX1_Control). (A) UMAP plot and pseudotime trajectory analysis of PDX1_Tam (green) and PDX1_Control tumors (red) (11705 cells). **(B)** *ESR1* gene expression. Note that the pseudotime trajectory from (A) bridges closely-associated *ESR1*-low cell clusters between PDX1_Control and PDX1_Tam tumors, suggesting that tamoxifen resistance in PDX1_Tam may originate from its *ESR1*-low cell cluster. **(C)** Seurat clusters. **(D)** Proliferation score. **(E)** Epithelial-like cancer stem cell (CSC) score. **(F-H)** Comparison of ARCHS[4] kinase and transcription factor scores along the pseudotime trajectory. Paths of interest are: between *ESR1*-high and -low PDX1_Control clusters (F); the "bridge" between *ESR*-low clusters from PDX1_Control and PDX1_Tam (G); and the three trajectory paths from the ESR1-low cell cluster to distinct *ESR1*-high cell clusters (H). Note that *ESR1*-related activity is restored across the "bridge" independently of *ESR1* expression.

Conclusions

- There is a conserved correlation of activity related to kinases such as *PIM3*, *IRAK2*, and *IGFR1* with cellular *ESR1* expression within ER+ tumors.
- *ESR1*-low clusters in both untreated and tamoxifen-treated PDX1 models are closely linked in UMAP space and pseudotime, with the pseudotime trajectory leading from the *ESR1*-low to three *ESR1*-high tamoxifen-resistant cell clusters.
- Kinases like *PIM3* and *ERBB2* may support *ESR1*-low cells in the tamoxifen-treated PDX1 model and permit activation of alternative pathways, potentially leading to evolving tamoxifen-resistant cell populations that eventually recover *ESR1* expression.

ESR1-low cells demonstrate consistent enrichment of kinase signatures and may represent a "funnel point" that seeds evolutionary trajectories of ET resistance

Investigating _ESR1_-low cell populations in ER+ tumors may yield novel and potentially actionable insights regarding early mechanisms of evolving endocrine therapy resistance.

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Contact

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