Transcriptomic regulation by pregnane x receptor drives pro-migratory signaling in human breast cancer cells

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Abstract

The expression and activation of the nuclear receptor Pregnane X Receptor (PXR) has been linked with poor outcomes in human breast cancer. While PXR's canonical role in drug metabolism is well defined in the liver and gastrointestinal tract, its contribution to tumor progression is less understood. We sought to define transcriptomic regulation mediated by PXR in breast cancer cells and its potential impact on metastasis. Triple-negative MDA-MB-231 cells were engineered to stably overexpress human PXR and treated with the agonist rifampicin. RNA-seq analysis revealed significant transcriptional changes compared with controls. KEGG and Gene Ontology enrichment identified pathways associated with cytoskeleton remodeling, adhesion complex components, and motility regulators. A panel of ten genes previously implicated in cancer cell migration was consistently up regulated ($log_2FC > 1.7$; $p < 0.05$). Post-hoc qPCR validation confirmed these results. Collectively, these data indicate that PXR expression enhances pro-migratory transcriptional pathways in breast tumor cells, providing mechanistic insight into its potential role as a driver of breast cancer metastasis and contributor to poor prognosis.

Introduction

The Pregnane X Receptor (PXR, NR1I2) is a ligand-activated nuclear receptor with a well-established role in regulating xenobiotic metabolism. Although PXR has been shown to be expressed in human breast tumors and has been associated with worse clinical outcomes, its molecular functions within breast cancer cells remain unclear. Furthermore, the specific transcriptional programs governed by PXR in this setting and their potential impact on tumor cell behavior have not been fully characterized.

In order to determine and define the roles of PXR expression and activation in human breast cancer, we generated stable PXR-overexpressing MDA-MB-231 cell lines and employed RNA-sequencing to identify ligand-dependent transcriptional changes. Follow-up gene expression validation, pathway enrichment, and functional assays were carried out to determine whether the transcriptional responses observed after PXR activation correlated with measurable phenotypic responses.

Methods

Full-length human PXR cDNA was subcloned into the pcDNA4/HisMax vector and used to generate stable PXR-overexpressing MDA-MB-231 cell lines through Zeocin selection, with expression confirmed by immunoblotting, immunofluorescence, and qPCR. Wild-type and PXR-overexpressing cells were treated with rifampicin (10 µM) or vehicle for transcriptomic and functional analyses. RNA-seq was performed to identify differentially expressed genes, followed by KEGG and Gene Ontology enrichment to define PXR-dependent pathways. Ten migration-associated transcripts emerging from RNA-seq were validated by quantitative RT-PCR. For functional assessment, pre-formed spheroids from wild-type and PXR-overexpressing cells were embedded in growth factor–reduced Matrigel and maintained with vehicle or rifampicin for one week. Invasive outgrowth was assessed using brightfield microscopy, phalloidin-stained Z-stacks, and ImageJ-based segmentation with convex-hull perimeter measurements to generate a perimeter/convex-hull ratio as an index of 3D invasion.

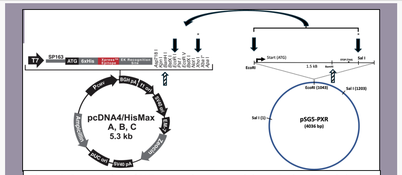


Figure 1. Construction of PXR expression vector. Schematic showing subcloning of full-length human PXR cDNA from pSG5-PXR into pcDNA4/HisMax using EcoRI and SalI restriction sites. The resulting construct (pcDNA4/HisMax-PXR) introduces an N-terminal Xpress/His epitope tag and Zeocin resistance for stable selection in mammalian cells.

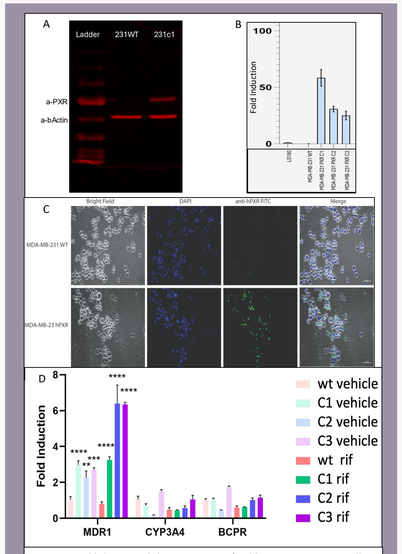


Figure 2. Establishment and characterization of stable MDA-MB-231 PXR cell lines. (A) Immunoblot of PXR and β-actin in wild-type (WT) and PXR-overexpressing (231c1) cells confirms robust PXR expression. (B) Quantitative RT-PCR validation showing fold-induction of PXR mRNA in multiple MDA-MB-231 PXR clones relative to WT and LS180 positive-control cells. (C) Immunofluorescence staining with anti-hPXR antibody (FITC) demonstrates strong nuclear PXR signal in transfected cells. (D) qPCR analysis of canonical PXR targets (MDR1, CYP3A4, BCPR) following 24 h rifampicin (10 µM) treatment; PXR-overexpressing clones display ligand-dependent induction of MDR1 expression. ****$p < 0.0001$.

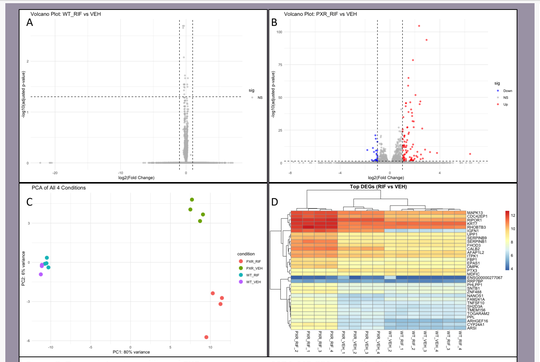


Figure 3. Transcriptomic profiling of PXR-overexpressing MDA-MB-231 cells. (A–B) Volcano plots comparing rifampicin vs. vehicle in WT (A) and PXR-OE (B) cells; significant DEGs are highlighted (red = up, blue = down, adj p < 0.05). (C) Principal component analysis (PCA) of all four groups (WT Veh, WT Rif, PXR Veh, PXR Rif) showing distinct clustering by PXR expression and treatment. (D) Heatmap of the top DEGs (Rif vs Veh) illustrating reproducible transcriptional changes among biological replicates.

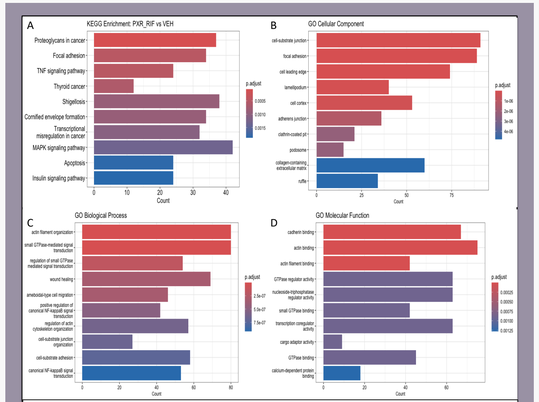


Figure 4. Functional enrichment of rifampicin-responsive genes in PXR-overexpressing cells. (A) KEGG pathway analysis identifies enrichment in cancer-related and signaling pathways including proteoglycans in cancer, focal adhesion, TNF, and MAPK signaling. (B–D) Gene Ontology enrichment shows strong association of DEGs with cell-substrate junctions, focal adhesion, actin filament organization, and GTPase-mediated signaling, consistent with PXR-linked regulation of cytoskeletal remodeling and cell migration.

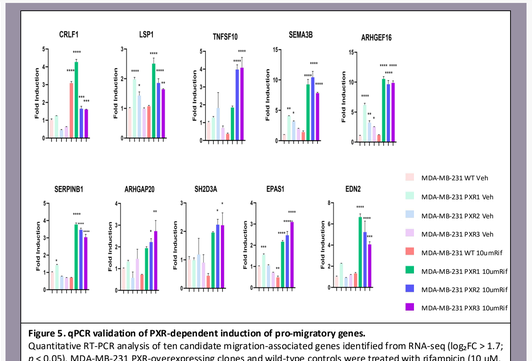


Figure 5. qPCR validation of PXR-dependent induction of pro-migratory genes. Quantitative RT-PCR analysis of ten candidate migration-associated genes identified from RNA-seq ($log_2FC > 1.7$; $p < 0.05$). MDA-MB-231 PXR-overexpressing clones and wild-type controls were treated with rifampicin (10 µM, 24 h). All genes demonstrated significantly elevated expression in PXR-overexpressing cells following ligand activation, consistent with transcriptomic predictions. Data represent mean ± SEM from biological replicates; significance determined by one-way ANOVA with post-hoc testing (**$p < 0.001$; ***$p < 0.0001$).

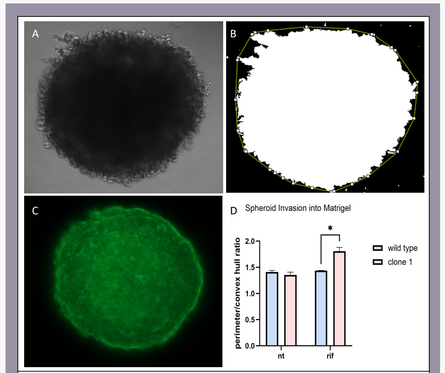


Figure 6. Ligand activation of PXR enhances 3D invasive outgrowth of MDA-MB-231 spheroids. Pre-formed wild-type and PXR-overexpressing MDA-MB-231 spheroids were embedded in growth factor–reduced Matrigel and treated with vehicle or rifampicin (10 µM) for one week. (A) Representative brightfield image of a PXR-overexpressing spheroid following rifampicin treatment. (B) Corresponding 8-bit ImageJ mask with convex-hull boundary applied for perimeter quantification. (C) Phalloidin-stained Z-stack fluorescence image highlighting actin-rich invasive structures. (D) Quantification shows that activation of PXR significantly increases invasive outgrowth compared with wild-type cells ($p < 0.05$).

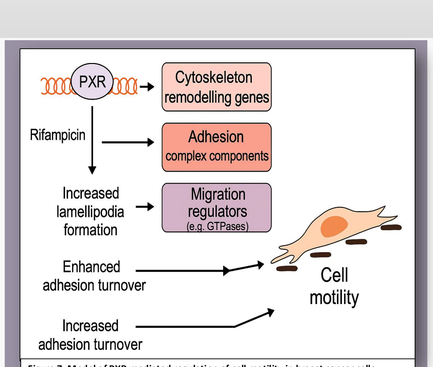


Figure 7. Model of PXR-mediated regulation of cell motility in breast cancer cells. Proposed model illustrating how activation of PXR by rifampicin induces transcription of genes involved in cytoskeletal remodeling, adhesion complex turnover, and migration regulation. These coordinated changes promote increased lamellipodia formation, enhanced adhesion dynamics, and overall elevated cell motility in PXR-overexpressing MDA-MB-231 cells.

Conclusions

Our results identify a previously unrecognized role for ligand-activated PXR in regulating transcriptional programs associated with motility and invasion in triple-negative breast cancer cells. RNA-seq analysis revealed that activation of PXR drives coordinated changes in genes linked to cytoskeletal remodeling, adhesion dynamics, and Rho GTPase signaling. qPCR validation confirmed ligand-dependent induction of multiple pro-migratory transcripts specifically in PXR-overexpressing cells, supporting a direct connection between PXR activation and these pathways. Functionally, PXR expression alone did not substantially alter invasive behavior; however, activation with a PXR agonist significantly increased 3D spheroid outgrowth into Matrigel, consistent with the transcriptomic signatures. Quantitative perimeter/convex-hull measurements and phalloidin imaging supported a ligand-dependent enhancement of invasive projections. These findings indicate that PXR activation promotes a pro-invasive phenotype in TNBC cells and suggest that exposure to endogenous, environmental, or therapeutic PXR ligands may influence tumor cell behavior and potentially contribute to disease progression.

Acknowledgements

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