Targeting ERβ signaling as a novel immunotherapeutic strategy in TNBC

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BACKGROUND

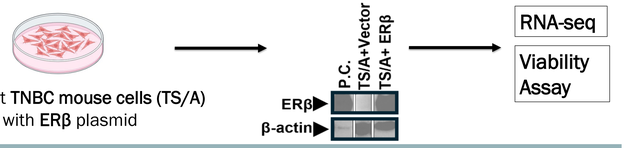
- **Triple Negative Breast Cancer (TNBC)** is a subtype of breast cancer possessing one of the poorest prognoses of all breast cancers.[1]
- Immunotherapy is a new treatment option but with **modest response rates.**
- **Recent studies using breast tumor samples show an association between the expression of estrogen receptor β (ERβ) and improved survival of patients with TNBC** and stronger adaptive immune-response programs.[2,3]
- **ERβ** - whose function largely opposes that of oncogenic ERα - is involved in the regulation of many tumor repressive pathways and has lately been observed to partake in immune modulation.[4]
- **ERβ thus presents a promising immunomodulatory target for TNBC.**

OBJECTIVE/HYPOTHESIS:

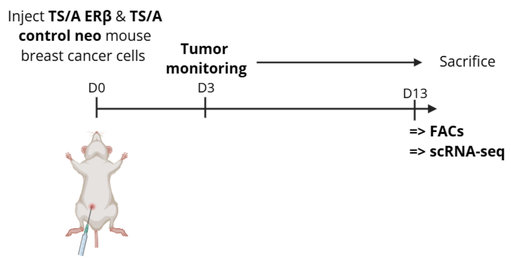
- We propose that activation of ERβ decreases the proliferative and metastatic ability of breast cancer cells by altering cellular pathways and crosstalk with immune cells.
- We generated a syngeneic mouse model to investigate the effects of ERβ on the tumor microenvironment (TME).

MATERIALS/METHODS

In vitro studies



In vivo studies



RESULTS

ERβ regulates expression of immune-related genes *in vitro* and inhibits tumor growth by altering the immunophenotype of immunocompetent mice

ERβ-expressing TS/A cell line

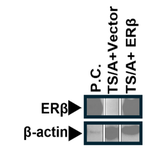


Figure 1: ERβ expression in control Neo (empty vector) and ERβ-expressing TS/A cells. P.C, positive control.

Tumor Growth Over Time

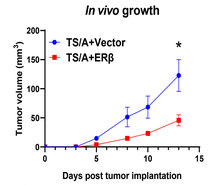


Figure 2: Tumor Growth Curve of orthotopically implanted TS/A ERβ vs. TS/A Neo tumors in a syngeneic mouse model.

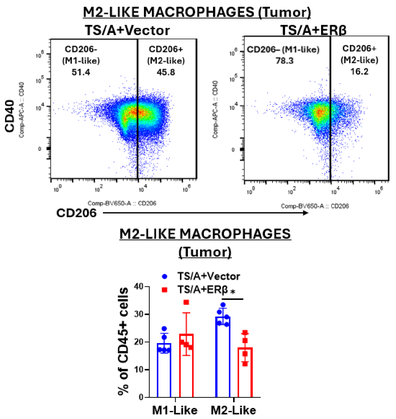


Figure 3: Flow Cytometry Analysis of the macrophage immunophenotype of control Neo and ERβ-expressing tumors.

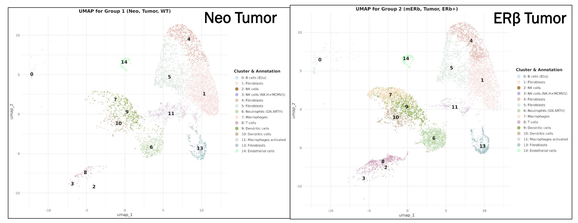


Figure 4: Single-cell RNA sequencing analysis shows ERβ-dependent changes in immune-cell proportions and activation states within the TME. ERβ-expressing tumors exhibit more M1-like macrophages whereas Neo control tumors exhibit a more M2-like profile.

ERβ Proliferation Assay

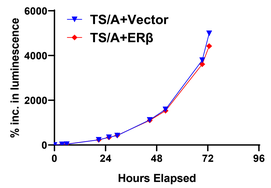


Figure 5: MT Viability Assay shows no difference in proliferation between TS/A Neo & TS/A ERβ.

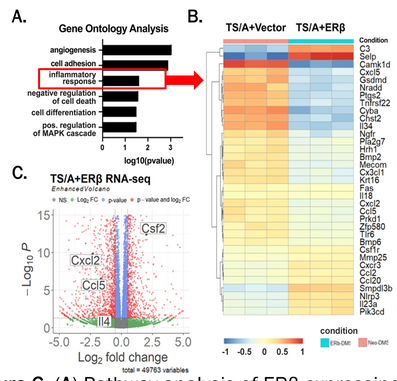


Figure 6. (**A**) Pathway analysis of ERβ-expressing TS/A cells reveals enrichment of the inflammatory response pathway and (**B**) the corresponding heatmap of related genes. (**C**) *In vitro* RNA-sequencing analysis of ERβ-expressing compared to control Neo TS/A cells demonstrates altered expression of key cytokines including CXCL2, CCL5, CSF2, and IL4.

Proposed Mechanism of Action

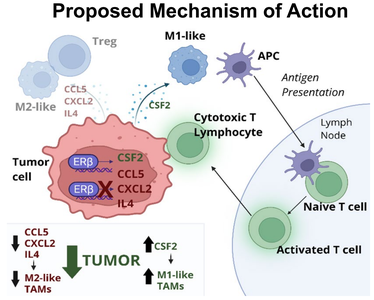


Figure 7. Schematic illustration of the mechanism of ERβ action on the TME of TNBC.

SUMMARY/SIGNIFICANCE

Here, we generated a syngeneic mouse model for studying the function of ERβ in the breast TME and showed that:

1. Tumor-specific ERβ leads to slowed tumor growth and a less immunosuppressive TME
2. Observed a reduction in tumor-associated M2-like macrophages upon upregulation of ERβ
3. Identified key target cytokines as downstream mediators that regulate macrophage function and antigen presentation

FUTURE ACTIONS

- **Delineate the function of ERβ** in the breast TME
- **Investigate clinical significance** by assessing correlation between ERβ expression and patient outcomes and biological markers
- **Evaluate potential ERβ agonists for therapy** by combining ERβ agonists with Immune Checkpoint Blockade and performing Pharmacokinetic and Toxicology studies

These studies help lay the groundwork for further research on the clinical potential of targeting estrogen receptor β (ERβ) as a treatment option for Triple Negative Breast Cancer (TNBC) and other subtypes lacking effective therapies.

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