‘Exploring the role of ERK5 in Triple Negative Breast Cancer’

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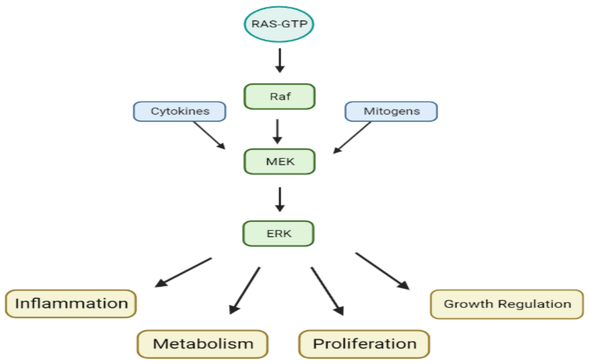
Abstract

Breast cancer is the most commonly diagnosed cancer among women and is classified by the presence of hormone receptors and human epidermal receptor 2 (HER2). Receptor positive breast cancers have available targeted therapies for treatment; however, the triple-negative breast cancer (TNBC) subtype lacks these receptors and to date has no targeted therapy. TNBC is a particularly aggressive, challenging to treat breast cancer, and is most prevalent in younger Black and Hispanic women, making this an underserved cancer. Due to the nature of the disease and its prevalence in minority populations, it is imperative to find effective novel targeted therapies.

One avenue researchers are interrogating for novel drug targets is through the exploration of protein kinases. Here, we focused on the extracellular signal-regulated kinase 5 (ERK5) a member of the MEK5/ERK5 pathway which regulates cellular proliferation, survival, differentiation, and apoptosis. The ERK5 pathway is known to have effects on TNBC, however the impact of this kinase on the tumor microenvironment (TME) is not currently evaluated.

ERK5 kinase evaluation was performed through the knockout (KO) of ERK5 in TNBC. Following validation of stable repression, conditioned media(CM) experiments were performed to understand the effects of ERK5 on the TNBC TME. Results demonstrated that MDA-MB-231-ERK5KO CM altered adipose derived stromal/stem cell (ASC) cytokine gene expression and cell death. Results from this study will be used to better understand the role of ERK5 in TNBC microenvironment and better inform novel drug targets.



Methodology

The MEK5/ERK5 pathway is a part of the mitogen activated protein kinase (MAPK) family, which has a well-defined role in oncogenesis. In TNBC specifically, ERK5 has been linked with drug resistance, metastatic progression, and more recently inflammation in tumors. TNBC is associated with markers for inflammation (IL8, IL6). Prior studies show that TNBC increases inflammation in ASCs. However, the role of ERK5 in ASC mediated inflammation is not known. The methods used here will use an ERK5 KO cell line (Figure 1A) in combination with CM experiments (Figure 1B) and qRT-PCR to determine if ERK5 is required for processes which drive inflammation in ASC through conditioned media experiments.

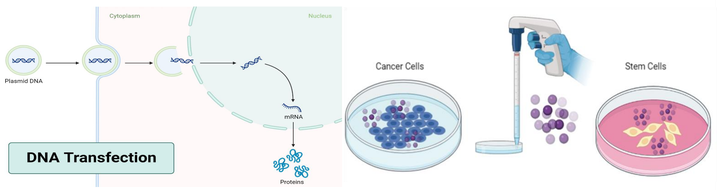
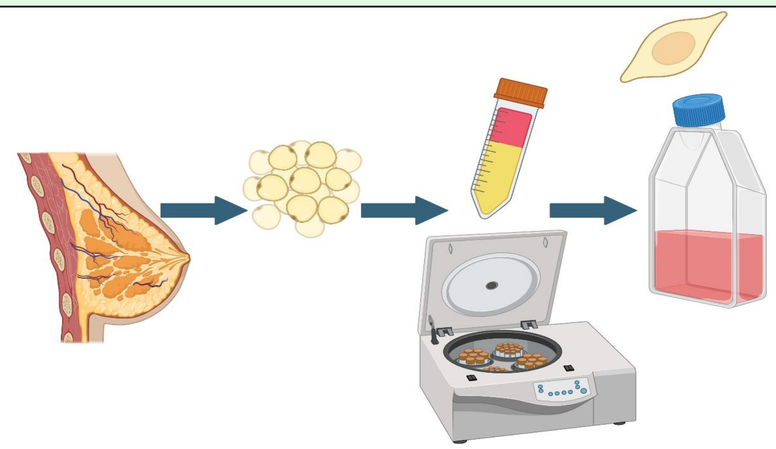


Figure 1. Overview of methods performed. (A) Transfection and gene expression overview (B) MDA-MB-231 and MDA-MB-ERK5KO condition media overview. (Both figures made with biorender)

Adipose Derived Stromal Cells



Adipose tissue is harvested subcutaneously from the breast after elective breast reduction surgeries from healthy individuals (IRB exemption). The tissue is minced and suspended in media, where the adipocytes float at the top, and the stromovascular fraction (SVF) falls to the bottom. The SVF contains immune cells, fibroblasts, and ASCs. SVF is seeded and cultured for retention of stromal cell populations. A total of n=2 donors were used for this study (Table 1).

Table 1. ASC Donor Information

ID	Tissue Source	BMI	Gender	Age	Race
326	Breast	31.70496	Female	52	Black
336	Breast	34.94201	Female	31	Black

ASC Cell Survival following Treatment with ERKKO Secretome

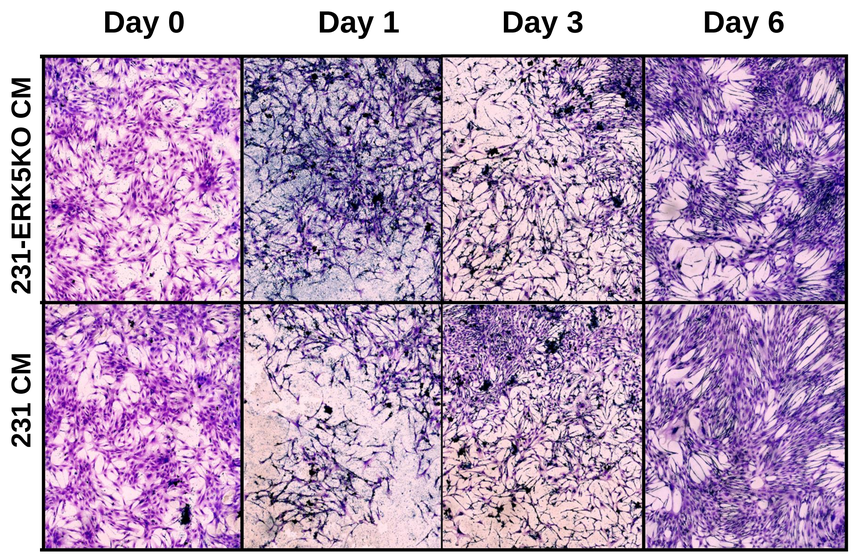


Figure 2. ASC growth is not altered by ERK5KO TNBC secretome. ASCs were seeded at 70% confluence and treated with CM form MDA-MB-231 or MDA-MB-231-ERK5KO cells. Cell number was evaluated at day 1, 3, and 6 with D0 control through crystal violet stain. n=1 ASC donors

ERK5 Secretome and TME Remodel

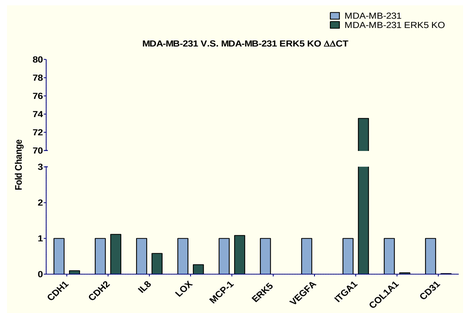


Figure 3. ERK5 KO Represses Genes Associated with TME. MDA-MB-231 and MDA-MB-231-ERK5KO cells were collected for total RNA extraction and qRT-PCR for genes associated with the TME. n=1 biological replicate. Housekeeping gene was beta actin.

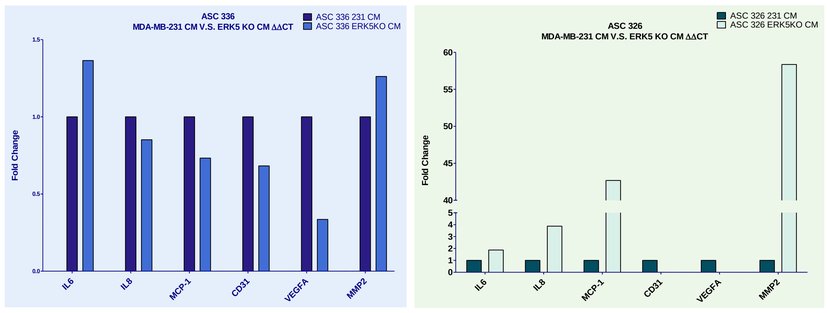


Figure 4. ERK5 KO Represses TME Genes in ASCs. (A-B) ASCs were stimulated with CM from MDA-MB-231 and MDA-MB-231-ERK5KO cells for 3 days. At end point, total RNA was extraction and qRT-PCR for genes associated with the TME. (A) ASC donor 1, with age of 31 (B) ASC donor 2 with age of 52. n=1 biological replicate per donor. Housekeeping gene was beta actin.

CDH1	**E-Cadherin** transcript and protein. Presents as an Epithelial marker.
IL-8	**Interleukin 8**. IL-8 (or CXCL8) is a chemokine that is related to inflammation and angiogenesis.
IL-6	**Interleukin 6 is a** pro-inflammatory cytokine and an anti-inflammatory myokine.
MMP2	**Mesenchymal (EMT) C**ell proliferation, inflammation, angiogenesis, and metastasis.
COL1A1	**Tumor architecture (collagen gene).** ERK5-ko cells showed down regulation of collagen genes.
LOX	(Lyzyl oxidase) **Matrix crosslinking** (crosslinks collagen) associated gene.
CD31	Angiogenesis.
MCP-1/CCL2	Monocyte Chemoattractant Protein-1. Key Chemokine that regulates migration of macrophages.

Conclusion & Future Directions

Conclusion

- ERK5KO suppresses TME associated genes in TNBC
- Secretome of ERK5KO in TNBC does not regulate ASC cell number
- ERK5 and the MEK pathway may alter ASC cytokine expression in specific donors
  - ERK5 KO inhibits ASC inflammation in donors with young patient age
  - ASC cytokine expression unaltered in ASC donors with increased age

Future Directions

- Due to the heterozygous nature of our donor population, the possibility of donor specific effects is likely. Widening the donor population would combat this.
- Additional studies on ASC donor age and ERK5 are necessary

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