Exogenous treatment with an inflammatory cytokine, Tumor Necrosis Factor Alpha, increases invasiveness in highly and weakly metastatic breast cancer cells MDA-MB-231 and MCF-7

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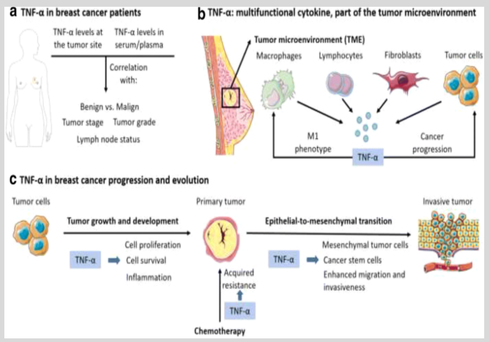
Introduction

- Breast cancer is a chronic disease that comprises 24.2% of total cancers and is the second leading cause of cancer mortality amongst women worldwide.
- Recent studies have shown that inflammation has been positively associated with the developmental progression of cancer.
- Tumor necrosis factor alpha (TNFα) is a multifunctional pro-inflammatory cytokine that regulates inflammatory responses as well as tissue remodeling.
- TNFα is also a prominent inflammatory mediator that promotes cancer cell invasion and metastasis initiating tumor promotion.
- Cancer metastasis may occur early in tumor progression is also associated with an increased risk of tumor recurrence and mortality representing one primary factor of cancer-related mortality.
- Inflammatory signals influence breast cancer progression, metastasis and therapeutic outcome by establishing a tumor supportive immune microenvironment.



Research Objectives

- This study aims to quantitatively compare the effect of TNFα treatment on cell invasiveness between MDA-MB-231 and MCF-7 breast cancer lines.
- **We hypothesize to see increased invasiveness in highly metastatic MDA-MB-231 cells compared to weakly metastatic MCF-7 cells and increased invasiveness of both breast cancer cell lines with the use of the inflammatory mediator TNFα.**

Methodology

The invasiveness of cells was evaluated under two conditions:
Cells were seeded in agarose gel with no additives or with 14.14ng/mL TNFα

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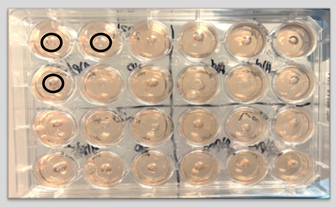
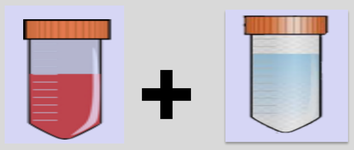
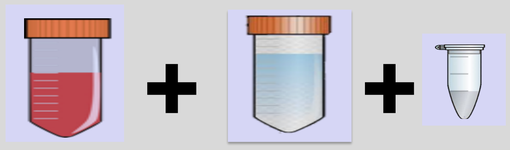
- To measure invasiveness, a gel matrix was made mixing 1% agarose gel with supplemented medium at a 1:1 ratio
- 1mL of solution was set in a 24 well plate
- Punches made in gel with a cutoff 1mL serological pipette





Supplemented Medium | **1% Agarose in PBS**



Supplemented Medium | **1% Agarose in PBS** | **14.14ng/mL TNFα**

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- Cells were harvested and suspended in serum free medium
- Cells were counted: 40,000 cells were seeded into each well
- Plate incubated under both conditions for seven days

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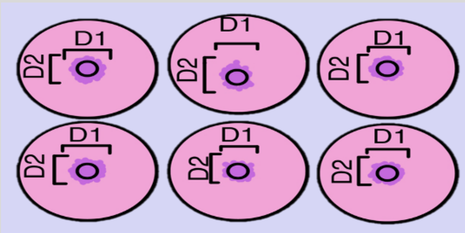
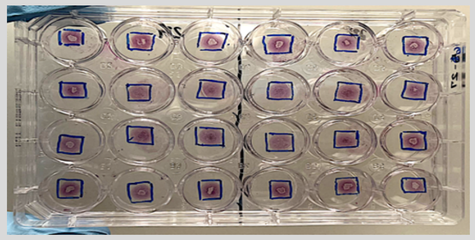
- Wells were fix with 10% Neutral buffer formalin solution for 15 minutes then washed with PBS
- Cells stained using 100µl of cresyl violet and sat overnight
- Cells were measured using the formula: **Area = π x (Diameter $_1$/2) x (Diameter $_2$/2)**





Results

t - test
n = 12
p < 0.05

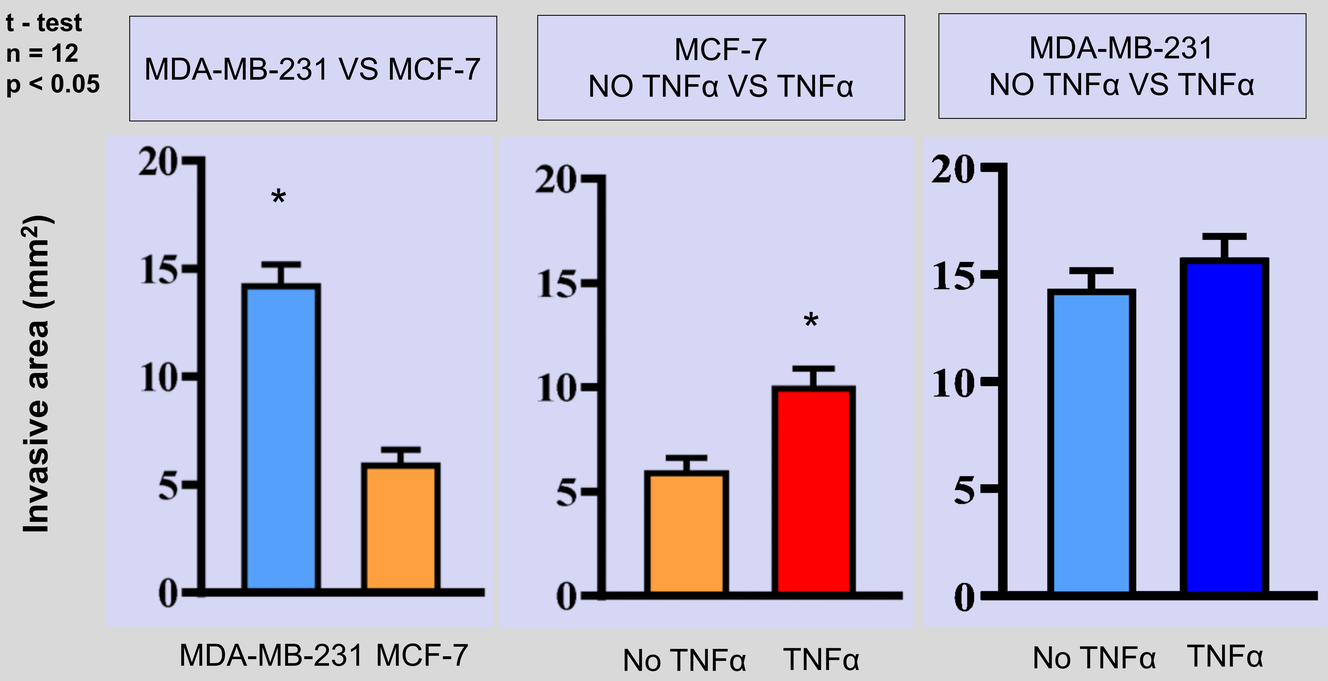


Figure 1.

- MDA-MB-231 is a highly metastatic breast cancer cell line, while MCF-7 is weakly metastatic.
- This graph showed significance that without TNFα, MDA-MB-231 is more invasive than MCF-7.

Figure 2.

- Because MCF-7 is the less invasive cell line, we wanted to know if adding TNFα would increase the invasiveness.
- MCF-7's invasiveness had a significant increase approximately 4mm^2.

Figure 3.

- MDA-MB-231 is a highly invasive breast cancer cell line.
- The addition of TNFα in the mixture did increase the invasiveness but did not show a significant difference.
- However, this is due to variabilities, so further studies are needed.

Conclusion

This experiment provides evidence that pro-inflammatory cytokine TNFα correlates positively with the increase of metastatic behavior in breast cancer. Overall, in this *in vitro* investigation, we confirmed our hypothesis to be true, to a certain extent.

- Highly metastatic MDA-MB-231 cells had increased invasiveness compared to MCF-7 cells.
- TNFα increased the invasiveness in the breast cancer lines, particularly the weakly metastatic MCF-7 cells.
- MDA-MB-231 followed a similar pattern, but the data gathered did not show a significant difference, so further studies are needed to provide a concrete conclusion.

Future Directions

- Suppressing pro-inflammatory cytokines to avoid systemic pro-inflammatory effects on breast cancer cells' invasiveness.
- Use anti-TNFα drugs to block inflammatory responses to decrease metastasis of cancer cells to avoid tumor promotion.
  - Conducting this study will allow us to determine whether ameliorating TNFα will decrease metastatic progression in breast cancer.

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