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

1. To measure invasiveness, a gel matrix was made mixing 1% agarose gel with supplemented medium at a 1:1 ratio
2. 1mL of solution was set in a 24 well plate
3. 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

- 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
- 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 (Diameter1/2) x (Diameter2/2)

Results

<table>
<thead>
<tr>
<th>t - test n = 12 p &lt; 0.05</th>
<th>MDA-MB-231 VS MCF-7</th>
<th>MCF-7 NO TNFα VS TNFα</th>
<th>MDA-MB-231 NO TNFα VS TNFα</th>
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<tbody>
<tr>
<td>Invasive area (mm²)</td>
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<tr>
<td>MDA-MB-231 MCF-7</td>
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<td>No TNFα TNFα</td>
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<td>Figure 1.</td>
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| • 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 4mm². |
| 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.

This research project was supported through the School of Graduate Studies and Oleander Medical Technologies.