The Effect of Levonorgestrel on Breast Cancer Progression

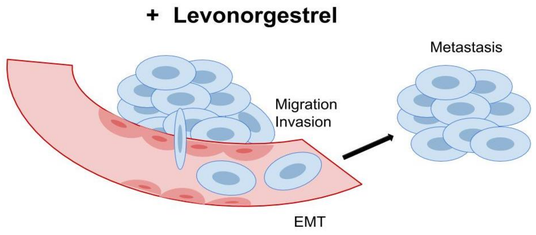
Victoria A. Moore[1], and Dr. Melissa N. Scheiber[1]
Department of Biology[1], Mary Baldwin University, Staunton, VA, 24401

Introduction

- Progesterone is an ovarian steroid hormone essential for normal breast development and its synthetic counterpart, progestin, is frequently prescribed as contraception (i.e. IUD's)
- 10.3% of females aged 15-49 use long-acting reversible contraceptives such as IUD's
- Levonorgestrel-releasing intrauterine system use has been associated with a higher incidence of breast cancer compared with the general population
- *In vivo* analysis of cancer progression allows a way to study levonorgestrel effects in an entire organismal system in addition to isolated *in vitro* conditions
- The role of levonorgestrel in breast cancer progression has yet to be elucidated

Hypothesis

It is hypothesized that levonorgestrel will enhance the progression of breast cancer through increased migration activity and gene regulation supporting epithelial-mesenchymal transition

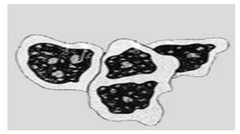


Acknowledgments

I would like to acknowledge Dr. Scheiber for her dedication, patience and support during this research. I would also like to thank the ABV Capstone committee and the Mary Baldwin University Biology Department for support and funding.

Experimental Design

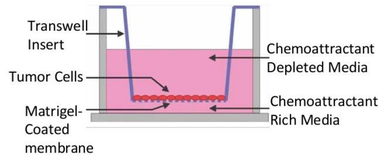
Cell Model
T47D cells
ER/PR +
Human ductal carcinoma



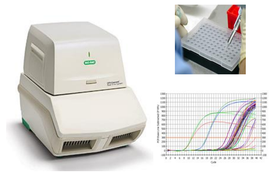
T47D cells

Experimental Groups
Untreated Control
DMSO Control
0.1 µM Levonorgestrel
0.01 µM β-estradiol
0.1 µM Lev. + 0.01 µM β-E2

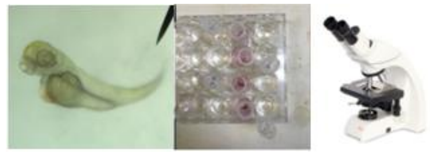
Migration Potential: Transwell® Migration Assay



RNA Expression: Real-Time RT-PCR



***In vivo* zebrafish embryo microinjection and cell quantification methods**



Results

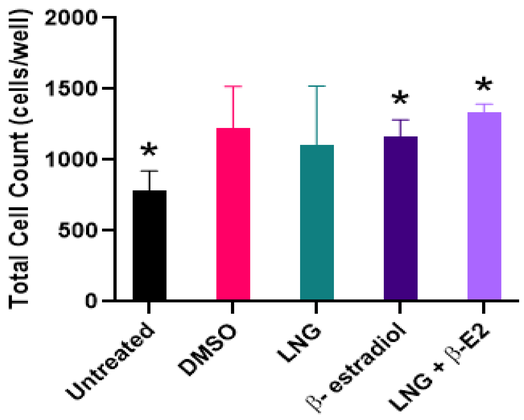


Figure 1. Migration analysis for T47D cells showed trend in increased migratory activity for β-estradiol and combined treatment group compared to untreated control, as determined by an unpaired t-test. (n=3, *P<0.05).

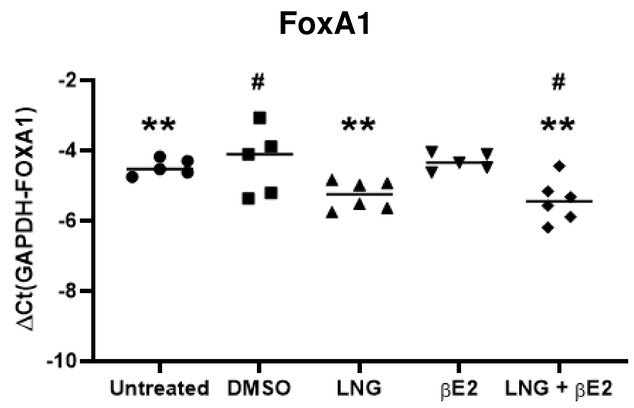


Figure 3. Real-Time RT-PCR analysis 72 hrs post treatment. Ct values are normalized to Ct values of GAPDH and plotted relative to untreated and DMSO controls. FoxA1 was significantly downregulated in the combined treatment group and showed a downregulatory trend in the levonorgestrel group, as determined by an unpaired t-test. (n=5-6, **P<0.01 to untreated, #P<0.05 to DMSO).

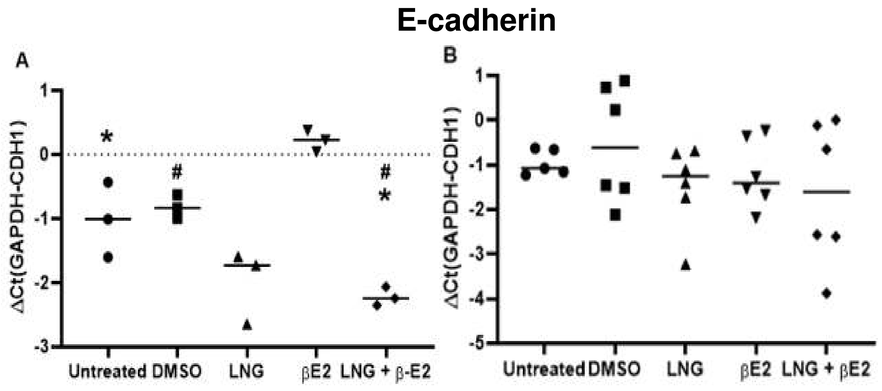


Figure 2. Real-Time RT-PCR analysis 72 hrs post treatment. Ct values are normalized to Ct values of GAPDH and plotted relative to untreated and DMSO controls. E-cadherin showed significant downregulation in the combined treatment group for run 1 (A), as determined by an unpaired t-test. (P<0.05, n=3-6). Run 2 showed a down-regulatory trend for the combined treatment group.

Conclusions

- Migration assays demonstrated a trend in increased migratory behavior when treated with β-estradiol and both levonorgestrel and β-estradiol for 72 hrs
- Real-Time RT-PCR revealed significant changes in FoxA1 genes after treatment, supporting its likely interaction with other epithelial-mesenchymal-associated genes
- Real-Time RT-PCR revealed trends in epithelial-mesenchymal-associated genes, E-cadherin, after treatment
- These results suggest that levonorgestrel in the presence of estrogen may induce an epithelial-to-mesenchymal transition and, therefore, enhance the metastatic potential of breast cancer

Future Directions

•Continue to observe the effects of levonorgestrel on metastatic potential *in-vitro* and *in-vivo*

•For the *in-vitro* models, further looking at the relationship between levonorgestrel and epithelial-mesenchymal-associated genes and repeating experiments at a greater incubation period to elicit more significant results

•For the *in-vivo* model, there has been successful optimization of immunochemical whole mount zebrafish staining of microinjected T47D cells to visualize cancer progression under light microscopy

References

- Daniels, K., & Abma, J. C. (2018). NCHS Data Brief: Current Contraceptive Status Among Women Aged 15-49: United States. 2015-2017., (327). Retrieved from https://www.cdc.gov/nchs/data/databriefs/db327_tables-508.pdf#3.
- Ruan X, Neubauer H, Yang Y, et al. Progestogens and membrane- initiated effects on the proliferation of human breast cancer cells. Climacteric 2012;15:467-72.
- Soini, T., Hurskainen, R., Grénman, S., Mäenpää, J., Paavonen, J., Joensuu, H., & Pukkala, E. (2015). Levonorgestrel-releasing intrauterine system and the risk of breast cancer: A nationwide cohort study. *Acta Oncologica, 55*(2), 188-192. doi:10.3109/0284186x.2015.1062538