The Effect of Levonorgestrel on Breast Cancer Progression

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In the United States, approximately 1 out of 8 women will develop invasive breast cancer over the course of her lifetime (U.S. Breast Cancer Statistics 2020). Many of these invasive breast cancers express the estrogen and/or progesterone hormone receptors (Ruan et al. 2012). Although numerous studies have described the carcinogenic role of estrogen in breast cancer, the role of progesterone has yet to be elucidated (Ruan et al. 2012). 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) (Daniels and Abma 2018). It was found that 10.3% of females aged 15-49 use long-acting reversible contraceptives such as IUD's (Daniels and Abma 2018). Specifically, the levonorgestrel-releasing intrauterine system use has been associated with a higher incidence of breast cancer compared with the general population (Soini et al. 2015).

Levonorgestrel, a synthetic progesterone found in birth control, was hypothesized to promote breast cancer progression through increased migration activity and gene regulation supporting epithelial-mesenchymal transition. An increase in migration activity as well as a decrease in epithelial-mesenchymal associated genes, FoxA1 and E-cadherin, would indicate a greater metastatic potential upon treatment of levonorgestrel. Epithelial-mesenchymal transition (EMT) is process where cells change their morphology and gene expression to assume the shape/characteristics of mesenchymal cells (Weinberg 2014). When this process is undergone in cancer cells, they translocate to other areas of the body, making the cancer metastatic and invasive (Weinberg 2014). FoxA1 and E-cadherin genes have been shown to be characteristic in

epithelial cells and when their expression becomes downregulated it indicates that the cells are likely undergoing epithelial-mesenchymal transition (BenAyed-Guerfali et al. 2019).

To test this hypothesis *in vitro*, progesterone receptor positive T47D breast cancer cells were analyzed under untreated control treatment, DMSO control treatment, 0.1 μM levonorgestrel treatment, 0.01 μM β- estradiol treatment, or 0.01 μM β- estradiol and 0.1 μM levonorgestrel combined treatment for 72 hours. These cultured cells were then analyzed via a migration assay as well as gene analysis through Real Time RT-PCR to understand migratory activity and gene expression related to epithelial-mesenchymal transition. Previous cellular viability assays demonstrated a significant increase in proliferation and no change in cellular death (Evans et al. 2019). Migration assay showed an increased trend of migration when cells were treated with β-estradiol or both β- estradiol and levonorgestrel. Real-Time RT-PCR revealed significant downregulation in epithelial-to-mesenchymal-associated genes, E-cadherin, and FoxA1.

Overall it was seen that the 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 decreases in FoxA1 genes after levonorgestrel and β-estradiol treatment, supporting its likely interaction with other epithelial-mesenchymal-associated genes. Real-Time RT-PCR also revealed decreased trends in epithelial-mesenchymal-associated gene, 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 studies will continue to investigate the effects of levonorgestrel on breast cancer progression, using both an *in-vitro* and *in-vivo* model. This can be done by continuing to observe the effects of levonorgestrel on metastatic potential *in-vitro* and *in-vivo.* Specifically, *in-vitro*,

further looking at the relationship between levonorgestrel and epithelial-mesenchymal-associated genes and repeating experiments at a greater incubation periods to elicit more significant results. *In vivo*, applications were done in zebrafish embryos that were microinjected with T47D cells and incubated with various treatments for 4 days post injection. There was successful optimization of a method involving immunochemical whole mount zebrafish staining of microinjected T47D cells to visualize cancer progression under light microscopy. This methodology allows for differentially staining of the cancer cells without cross reacting with zebrafish cells, which enables future studies to quantify the effect of levonorgestrel on cancer cell progression *in vivo*.

References

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