THE ROLE OF ESTROGEN IN PROMOTING TRIPLE NEGATIVE BREAST CANCER METASTASES IN THE BRAIN

Pauline Hoosepian-Mer, M.S., M.D. Candidate
With Diana Cittelly, PhD (PI) and Maria Jose Contreras-Zarate, PhD, Department of Pathology
University of Colorado Anschutz Medical Campus

ABSTRACT

Brain metastases (BM) from breast cancer portend a poor prognosis and a challenge to therapies. BM affect pre-menopausal women disproportionately and are more common in triple- negative (TN) breast cancers (TNBC), lacking estrogen receptor alpha, progesterone receptor, and HER2. Previous studies in the Cittelly lab[1] demonstrated a role of estrogen in mediating breast cancer metastases in the brain through the upregulation of brain-derived neurotrophic factor (BDNF) via estradiol (E2)-stimulated astrocytes and subsequent activation of tropomyosin kinase receptor B (TrkB) in tumor cells. This project aims to:

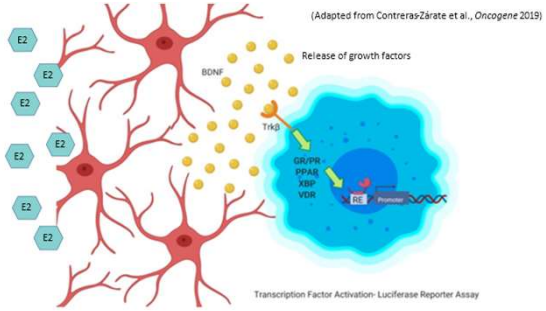
1) **Explore** whether E2-depletion therapies can reduce brain metastatic burden
2) **Elucidate** transcription factor downstream of BDNF/TrkB which ultimately induce transcription to promote metastases in triple negative breast cancer cell lines.
3) **Describe** microglial recruitment and activation profiles at metastatic foci

To determine the role of estrogen-depletion to curtail/reduce metastatic burden in an in-vivo model, qRT-PCR was conducted from DNA extracted from mouse brain hemispheres to quantify mCherry-expressing brain metastases. To investigate the activation of transcription factors glucocorticoid receptor/progesterone receptor (GR/PR) and peroxisome proliferator-activated receptor-α (PPAR) downstream of BDNF/TrkB, reporter gene assays were conducted using luciferase reporter plasmids transfected into triple negative cancer cell lines, MDA-MB-231 and BM-PDX-F2-7. Immunofluorescence staining was conducted using primary antibodies for TMEM 119 (general for microglia), CD16/32 (M1-profile microglia), and CD206 (M2-profile) to describe brain metastatic foci microglia recruitment and activation profiles.

Together, these aims will contribute to define the role of estrogen in the brain niche and the potential to integrate estrogen-depletion or TrkB inhibitors in metastatic breast cancer—to inform best practices and improve patient outcomes.

INTRODUCTION

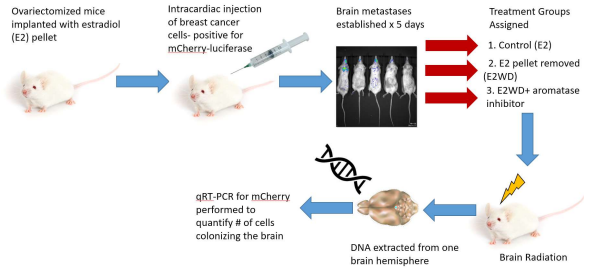
Symptomatic brain metastases (BM) develop in 10–16% of patients with metastatic breast cancer. Prognosis of these patients is dismal with a survival varying from 4 to 18 months with multimodal therapies [2]. BM affect pre-menopausal women disproportionately with the incidence 53% versus 28% in postmenopausal women [3]. Also, BM are more common in triple- negative (TN) breast cancers (TNBC), lacking estrogen receptor alpha, progesterone receptor, and HER2 [4].



Contreras-Zarate et al. investigated the role of estrogen in priming the brain niche for breast cancer metastases via paracrine signaling promoted by activated astrocytes and upregulation of brain-derived neurotrophic factor (BDNF). The subsequent activation of tropomyosin kinase receptor B (TrkB) in tumor cells promoted invasion, survival, and colonization via transcription factor activation. GR regulates many genes culminating in inflammation, catabolism, apoptosis and cell survival. In ER- negative breast cancer including TNBC, high tumor expression of GR correlates with higher tumorigenicity, aggressiveness, chemotherapy-resistance, and overall worse prognosis [7]. Activated microglia have been identified surrounding metastatic loci. The classical activation of macrophages, M1 activated, has been described as the pro-inflammatory phenotype which is anti-tumorigenic in effect, while the alternative activation profile, M2, is described as anti-inflammatory and, ultimately, tumor-supporting [8].

METHODS

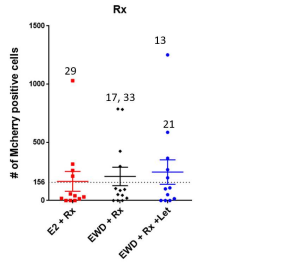
1) Can estrogen-withdrawal reduce brain metastases in a clinical setting (established mets + radiation)?



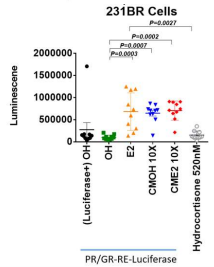
2) Reporter gene assays using luciferase plasmids MDA-MB-231 and BM-PDX-F2-7

3) Immunofluorescence for TMEM 119 (general for microglia), CD16/32 (M1-profile microglia), and CD206 (M2-profile)

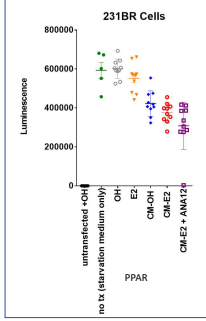
RESULTS



- Radiation dramatically altered brain metastatic growth
- Low tumor burden in all treatment groups (0 to 1000 metastatic cells per hemisphere despite expected metastatic burden of1000-20000 cells per hemisphere)
- No statistically significant difference (α=0.05)



- Successfully transfected 231BR cell line
- Estrogen alone (E2, conditioned media from astrocytes induced by estrogen (CM-E2), or conditioned media from astrocytes with vehicle (CM-OH) had similar luminescence
- E2 alone increased transcription in TNBC



- For PPAR, conditioned media groups previously demonstrating increased TF activation showed lower luminescence
- ANA-12 treatment group had the lowest luminescence, which was consistent with its effects of blocking BDNF-induced downstream pathways as a selective non-competitive antagonist of TrkB

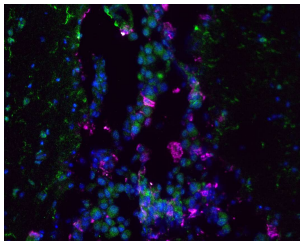
CONCLUSIONS AND DISCUSSION

Radiation therapy at our current setting resulted in drastic attenuation of metastatic burden, in micro-metastases (<300 µm), yielding differences in qPCR too subtle to ascertain treatment differences requiring future experiments in radiation treatment adjustments, immunohistochemistry/immunofluorescence validation, or investigation in a larger or different tumor model (in process in syngeneic animal model). Future studies in radiation settings and the optimal therapeutic window warrant further investigation in targeted therapies aimed at relieving tumor burden. In a syngeneic model, there was no statistical difference in treatment arms with significant level α=0.05; however, hypothetically, to address underpowered study due to limited sample size (n=36) a significant level of 10% would render statistically significant difference between E2 and EWD+letrozole samples—affording credence to a potentially highly relevant clinical difference.

The reporter gene assay outcomes were performed and optimized by two separate lab personnel. Concerns about yield and toxicity from the transfection were raised over the course of multiple experiments. MDA-MB-231 was shown to be highly sensitive to toxic compounds, which may have been introduced in the transfection reagents resulting in cellular damage and diminished reporter gene expression [9]. Cell-based reporter gene assays are inherently difficult due to large number of potential targets, high level of crosstalk among signaling pathways which are difficult to narrow and confirm [10].

Furthermore, estradiol alone increased transcription in TNBC warranting investigation into the role of estrogen receptor beta, which has a previously established non-tumorigenic profile. The inactivate state of PPAR is attributed to the pro-oncogenic profile [11]in TNBC, and evidently, the activation may inhibit metastasis explaining the presumably contradictory decrease in luminescence. Exploring PPAR agonism may be a therapeutic potential.

FUTURE DIRECTIONS



- Identify and validate brain metastases and describe microglial subtypes (pro- versus -anti-inflammatory) within tumor foci in triple negative breast cancer cell lines
- Syngeneic murine model was used to determine the ability of TNBC cell line to colonize the brain in the same inbred, immunocompetent mouse strain as the sourced tumor cells

* TMEM119 (Green), not common to bone-marrow derived macrophages [12] labels resident microglia

* Increased microglial concentration suggests recruitment in sections with confirmed brain metastases with M2-profile (Magenta) reliably present

* Continue to determine M1/M2 profile and the role of estrogen in promoting microglial recruitment and effects on metastatic burden

REFERENCES

1. Contreras-Zárate MJ et al. Estradiol induces BDNF/TrkB signaling in triple-negative breast cancer to promote brain metastases. Oncogene. 2019 Feb 22.
2. Sartorius CA et al. Estrogen promotes the brain metastatic colonization of triple negative breast cancer cells via an astrocyte-mediated paracrine mechanism. Oncogene. 2016;35(22):2881–92.
3. Hung MH, et al. Effect of age and biological subtype on the risk and timing of brain metastasis in breast cancer patients. PLoS ONE. 2014;9: e89389.
4. Morris PG et al. Limited overall survival in patients with brain metastases from triple negative breast cancer. Breast J. 2012 Jul-Aug;18(4):345-50.
5. Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol. 2001;63: 71–124. 43.
6. Cunha C, Brambilla R, Thomas KL. A simple role for BDNF in learning and memory? Front Mol Neurosci. 2010;3:1.
7. Pan D, Kocherginsky M, Conzen SD. Activation of the glucocorticoid receptor is associated with poor prognosis in estrogen receptor-negative breast cancer. Cancer Res. 2011;71:6360–6370.
8. Pukrop T, Dehghani F et al. (2010). Microglia promote colonization of brain tissue by breast cancer cells in a Wnt-dependent way. Glia 58 1477–1489. 10.1002/glia.21022
9. Kretzer, N. M. et al. A Noncompetitive Small Molecule Inhibitor of Estrogen-Regulated Gene Expression and Breast Cancer Cell Growth That Enhances Proteasome-Dependent Degradation of Estrogen Receptor α. J. Biol. Chem. 2010, 285, 41863–41873.
10. Liu AM, New DC, Lo RK, Wong YH. Reporter gene assays. Methods Mol Biol. 2009;486:109-23.
11. Wang Y, Zhu M, Yuan B , Zhang K, Zhong M, Yi W, Xu X, Duan X. VSP-17, a New PPARγ Agonist, Suppresses the Metastasis of Triple-Negative Breast Cancer via Upregulating the Expression of E-Cadherin. Molecules. 2018; 23(1):121.
12. Bennett M. L., et al. New tools for studying microglia in the mouse and human CNS. Proc. Natl. Acad. Sci. U.S.A.