

Abstract

THE ROLE OF ESTROGEN IN THE BRAIN MICROENVIRONMENT IN PROMOTING TRIPLE NEGATIVE BREAST CANCER METASTASES. PA Hoosepian-Mer, (M.D., G.S.), MJ Contreras-Zarate, and DM Cittel, Department of Pathology, University of Colorado School of Medicine, Aurora, CO.

Background:

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 Cittel lab demonstrated a role of astrocytes 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.

Aims:

This project aims to 1) define whether E2-depletion therapies can reduce brain metastatic burden in a clinical setting, 2) elucidate transcription factors downstream of BDNF/TrkB which ultimately induce transcription to promote metastases in triple negative breast cancer cell lines, and 3) describe microglial recruitment and activation profiles within the brain tumor microenvironment. 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.

Methods:

To determine the role of estrogen-depletion as a means 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 after single dose radiation treatment. 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. To describe microglia recruitment and activation profiles at metastatic foci, immunofluorescence staining was conducted using primary antibodies for TMEM 119 (general for microglia), CD16/32 (M1-profile microglia), and CD206 (M2-profile).

Results:

Radiation altered dramatically brain metastatic growth in this model, with low tumor burden in all treatment groups (0 to 1000 metastatic cells per hemisphere, expected metastatic burden is 1000-20000 cells per hemisphere) at this time point). This preliminary study did not show any additional effect of E2WD or E2WD+ AIs in metastatic burden; warranting immunohistochemistry confirmation of brain metastases in future experiments, as well as further studies at lower radiation doses. Preliminary results revealed the greater transfection efficiency in 231BR cells over patient-derived cell line F2-7. Also, positive controls for glucocorticoid receptor and progesterone receptor verified some specificity of our reporter gene system; however, estradiol alone

increased transcription in TNBC warranting investigation into the role of estrogen receptor beta, which has a previously established non-tumorigenic profile.

Conclusions:

Micro-metastases measured via qPCR did not significantly differ within treatment arms, requiring further assessment of radiation strength, early versus late treatment window optimization, and immunohistochemistry and immunofluorescence validation. Ongoing in-vitro research into the role of transcription factor investigation continues in breast cancer cell lines treated with conditioned media derived from estradiol-stimulated astrocytes with the ultimate goal to elucidate the downstream biochemical mechanisms involved in promoting metastases.