Transcription factors that modulate adaptation of Triple Negative Breast Cancer cells to the brain niche

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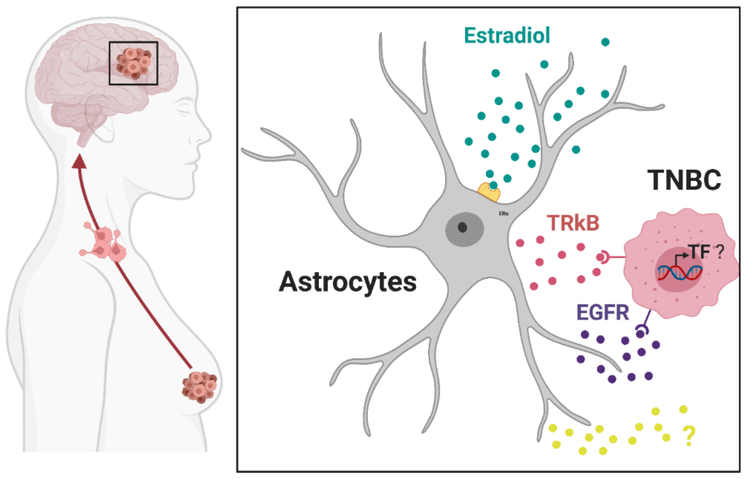
Introduction

- Brain metastasis (BM) occur in 30-50% of women with metastatic Triple Negative Breast Cancer (TBNC). The incidence of BM is higher in pre-menopausal women (53%) compared to post-menopausal women (28%).
- We have previously shown that pre-menopausal levels of estradiol (E2) promotes TNBC BM by acting on ER+ astrocytes within the brain microenvironment. [1,2]
- E2-treated astrocytes increase proliferation and tumor-initiating ability of TNBC cells by activating EGFR and TRkB in a paracrine manner. [1]
- The downstream molecular mechanisms triggered by multiple astrocytic signals in cancer cells remains unknown. [2]

Hypothesis: Interaction of TNBC cells with E2 treated astrocytes leads to modulation of specific transcription factors that trigger the transition of cancer cells from dormancy to outgrowth.



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Results

Serum-free conditioned media from E2-treated astrocytes results in nuclear enrichment of multiple transcription factors in 231BR and F2-7 cells

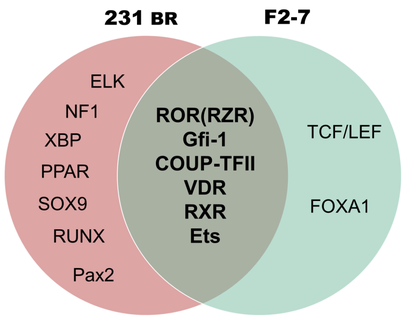

Figure 1. Conditioned media from E2-tretated astrocytes leads to nuclear enrichment of transcription factors in TNBC cells. 231BR and F2-7 cells were treated 1 hour with Vehicle (Ethanol), serum-free conditioned media of estrogen-treated astrocytes (CM-E2) or conditional media of vehicle-treated astrocytes (CM-OH) following 12 hours with starvation media. Nuclear extracts were isolated and then analyzed in a transcription factor array using F Activation Profiling Plate Array II (Signosis, Inc. Cat #FA-1002). Venn diagram shows the transcription factors increased in the nuclei of 231 BR and F2-7 cells stimulated with conditioned media with estrogen.

COUP TFII forms homodimers or heterodimers to activate or repress gene expression

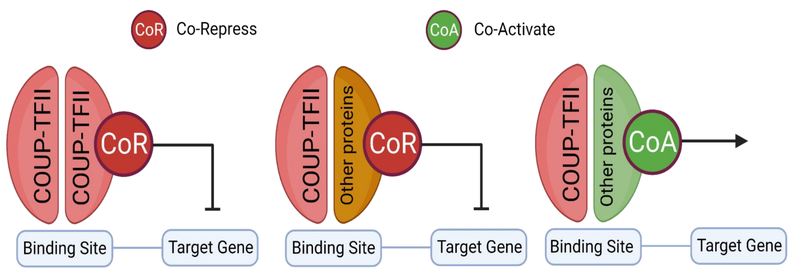

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Figure 2 Known mechanism of COUP-TFII transcriptional regulation. COUP-TFII may regulate downstream target gene expression directly (homodimer) or indirectly, through heterodimer formation with other proteins. When coupled with other proteins COUP-TFII can repress or activate the target gene. The binding site and the coupling partner depend on the cell environment.

Results

Clinical samples of brain metastasis from different subtypes of breast cancer express COUP TFII

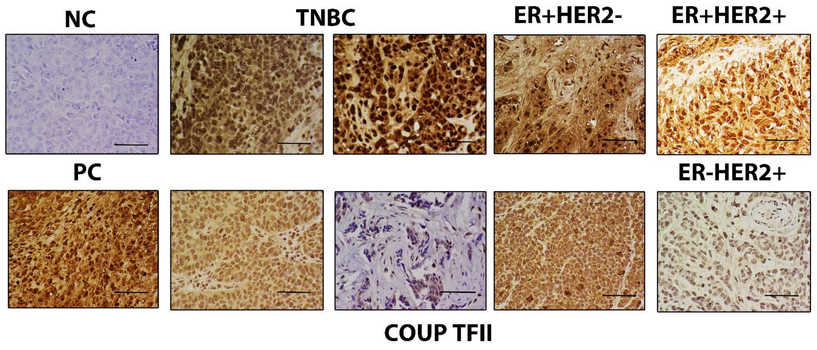

Figure 3. COUP TFII is present in brain metastasis from different subtypes of breast cancer. Immunohistochemistry staining for COUP TFII, using Mouse Anti-COUP-TFII 1:400 (R&D Cat#: PP-H7147-00) in brain metastasis patients PFA samples with different subtypes of breast cancer. Pictures were taken at 40x magnification. **NC** (Negative Control) mouse normal brain. **PS** (Positive Control) Human brain metastasis. All samples shown the presence of COUP TFII in nuclei and cytoplasmic.

Serum-free conditioned media from E2-treated astrocytes increase levels of COUP TFII in 231BR cells

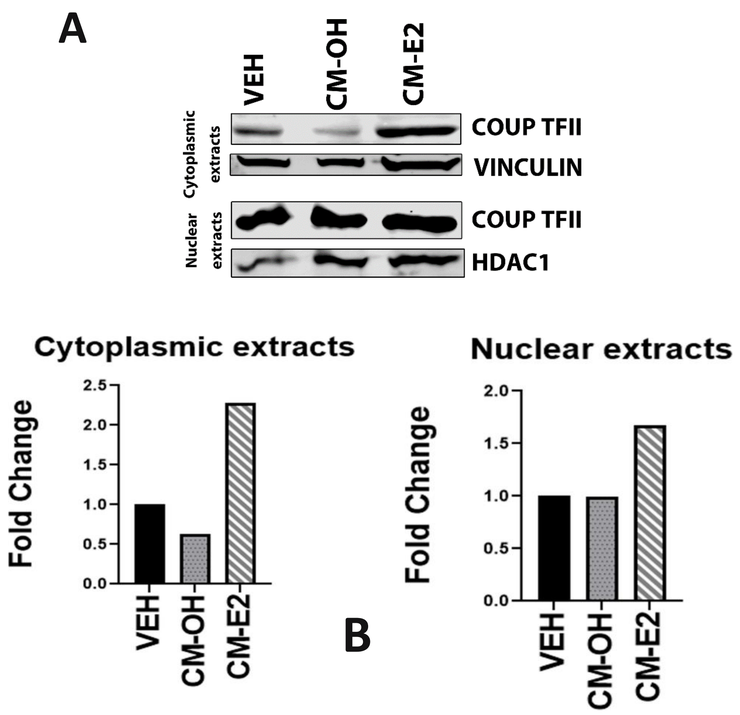

Figure 4. CM-E2 increase expression of COUP TFII in 231BR cells. (A) WB of COUP TFII in cytoplasmic and nuclear extracts of 231BR cells, treated 1 hour with Vehicle (Ethanol), astrocytes conditioned media with estrogen (CM-E2) and conditional media with vehicle (CM-OH). Vinculin was used as loading control for cytoplasmic extracts and HDAC1 was used as loading control for nuclear extracts. **(B)** Fold Change of COUP TFII levels relative to vehicle in cytoplasmic and nuclear extracts of 231 BR cells from B. Densitometry results were normalized to their respective loading control.

Serum-free conditioned media from E2-treated astrocytes increase levels of RORγ in 231BR cells

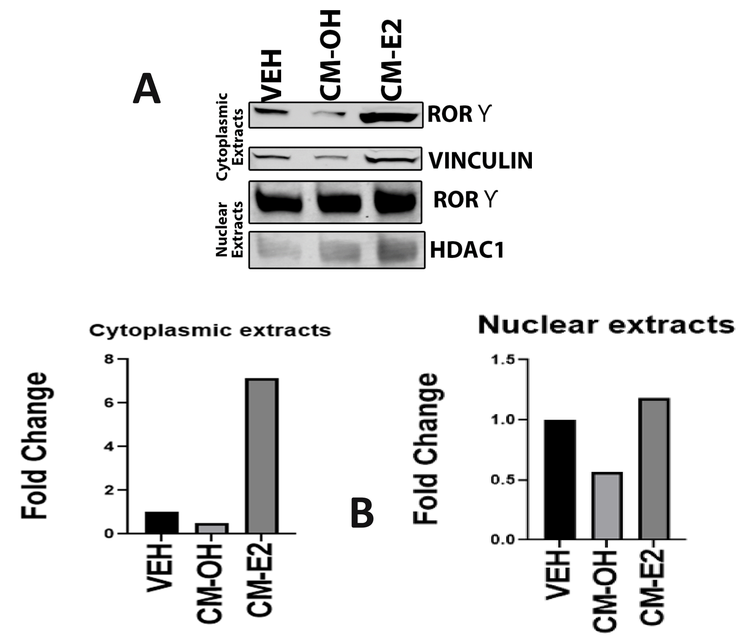

Figure 5. CM-E2 increase expression of RORγ in 231BR cells. (A) WB of RORγ in cytoplasmic and nuclear extracts of 231BR cells, treated 1 hour with Vehicle (Ethanol), or serum-free conditioned media of astrocytes treated with estrogen (CM-E2) or vehicle (CM-OH). Vinculin was used as loading control for cytoplasmic extracts and HDAC1 was used as loading control for nuclear extracts. **(B)** Fold Change of RORγ levels relative to vehicle in cytoplasmic and nuclear extracts of 231 BR cells from B. Densitometry results were normalized to their respective loading control.

Knockdown reduces levels of COUP TFII in 231BR cells

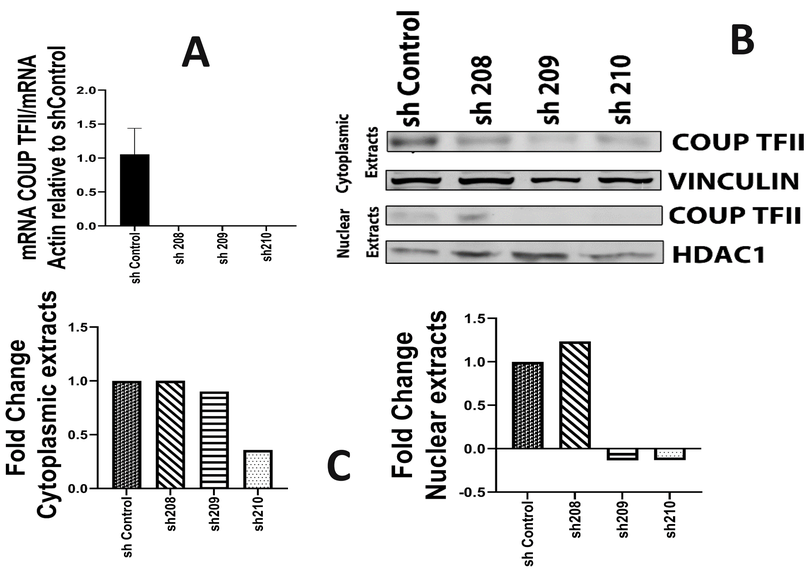

Figure 6. shRNA Knockdown reduces levels of COUP TFII in 231BR cells. (A). mRNA levels of COUP TFII isolated from 231BR cells with non-targeting control (sh Control) and three shRNAs for COUP TFII (sh 208, sh209 and sh210. Human β-actin was used for normalization. **(B).** WB of COUP TFII in cytoplasmic and nuclear extracts of 231BR cells as in A. Vinculin was used as loading control for cytoplasmic extracts and HDAC1 was used as loading control for nuclear extracts. **(C)** Fold Change of COUP TFII knockdown levels relative to control in cytoplasmic and nuclear extracts of 231 BR cells from B. Densitometry results were normalized to their respective loading control.

Conclusions & Future Directions

- *CME2 leads to nuclear recruitment of COUP TFII and RORγ in TNBC cells.*
- *shRNAs for knockdown COUP TFII deplete its gene expression and decrease the protein levels in 231BR cells, however, cells showed low growth and reduced viability, precluding functional assays.*
- *Currently developing an inducible system for temporal downregulation of Coup TF and RORγ.*
- *Determine open chromatin profiles of TNBC cells treated with vehicle vs CME2.*

References: 1. Sartorius CA, et al, Cittelly DM. Oncogene, 2016.
2. Contreras-Zarate et al, Cittelly DM. Oncogene, 2019

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