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Research from CleopatraRX inventor, Rhonda Voskuhl, MD, presented by Noriko Itoh.

Targeting estrogen receptor beta (ERβ) in astrocytes to prevent cognitive domain-specific deficits of menopause

Cognitive issues occur in 50-70% of menopausal women. Estrogens are known to have neuroprotective properties in several neurodegenerative disease models. However, the type and dose of estrogen to prevent menopause related cognitive decline remains unknown. Estradiol treatment in standard hormone replacement therapy (HRT) has some potential to counter deleterious effects of loss of estrogen during aging in women, but it is only FDA approved to treat hot flashes. Treatment is limited to relatively short durations at the lowest possible dose to minimize estradiol binding to estrogen receptor alpha in breast and risk for breast cancer. Thus, we pursued use of estrogen receptor beta (ERβ) ligands for neuroprotection during menopause given published neuroprotective effects in multiple sclerosis (MS) women and its mouse model.

Our earlier studies demonstrated that female mice with loss of ovarian hormones in midlife, but not young adulthood, had hippocampal-dependent cognitive impairment, and activation of astrocytes and microglia with synaptic loss. This was a sex hormone by age interaction. These pathologies also occurred in gonadally intact females with selective deletion of ERβ in astrocytes, but not neurons. RNA sequencing of hippocampal astrocyte-specific transcriptomes in the astrocyte ERβ CKO, compared to age-matched wildtypes, identified Gluconeogenesis I and Glycolysis I as the most differentially expressed pathways, with *Enolase 1* (*Eno1*) the most differentially expressed gene. Analyses of existing human datasets in menopausal women showed upregulation of human *ENO 1* gene expression.

Here, loss of functional studies in astrocyte ERβ CKO mice, compared to age-matched wildtypes, showed increased ENO1 at the protein level in hippocampal reactive astrocytes. Increased ENO1 protein expression was also observed in reactive astrocytes of ovariectomized midlife females. Importantly, gain-of-function studies demonstrated that treatment with an ERβ ligand in ovariectomized midlife female mice reversed the upregulation of ENO1 protein expression in hippocampal reactive astrocytes and improved cognitive function. These findings suggest therapeutic strategies to counter abnormalities in glucose metabolism in astrocytes by treatments stimulating ERβ in astrocytes.

Hypothetical Effect of Upregulated Enolase 1 in Astrocytes During Menopause. (A) During health, at young ages, astrocytes are a primary site for glycolysis and provide energy substrate (lactate) to neurons. **(B)** During midlife, either selective deletion of ERβ in astrocytes or depletion of endogenous estrogen leads to the upregulation of ENO1 in reactive astrocytes. This upregulation of ENO1 aligns with a metabolic shift away from glycolysis and toward gluconeogenesis with impaired glucose utilization. Impairment of glucose metabolism in brain of midlife women has been shown. Additionally, gluconeogenesis is known to occur in brain astrocytes. Together, our findings suggest that treatment with estriol may normalize ENO1

expression in reactive astrocytes with potential to reverse abnormal glucose utilization and improve both cognitive function and neuropathology in menopausal women.