Reproductive Endocrinology
CLINICAL STUDIES IN FEMALE REPRODUCTION I

SSRI Use in the Peripartum Period Regulates Mammary Gland Parathyroid Hormone Related Protein (PTHrP) by a Serotonylation-Dependent Mechanism

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During lactation, a woman experiences a considerable amount of bone loss and recent studies suggest bone deficits persist years postpartum. Furthermore, selective serotonin uptake inhibitors (SSRIs), which are often prescribed to women experiencing peripartum depression, have been linked to osteopenia. Serotonin signaling can increase parathyroid hormone related protein (PTHrP), a bone remodeling protein which liberates calcium for the milk. Additionally, fluoxetine (a common SSRI) results in increased mammary gland serotonin content and PTHrP, and treatment during the peripartum period reduced maternal bone mineral density. One proposed mechanism of serotonin action is by its covalent addition to proteins by transglutaminase (TG2), termed serotonylation. We therefore investigated whether the combination of fluoxetine and lactation can exacerbate maternal bone loss and the underlying mechanism. We hypothesized that SSRI-induced serotonin signaling in the lactating mammary gland increases PTHrP through a serotonylation-dependent mechanism. Treatment of mouse mammary epithelial cells (HC11) with fluoxetine significantly upregulates PTHrP gene expression and the concentration of its downstream effector, cAMP, over control (P < 0.0004). Furthermore, treatment of the HC11 cells with fluoxetine in addition to a TG2 inhibitor, monodansylcadaverine, restores PTHrP mRNA expression to levels observed in the control. Small g-proteins have emerged as a common target protein for serotonylation. Currently, our data suggest that the g-proteins, RhoA and Rab4, are potential serotonylation targets in the mammary gland. Together these data suggest that the molecular process of serotonylation in HC11 cells links serotonin signaling to increased PTHrP expression. Future work is directed at using the cre-lox system to genetically ablate serotonylation using a WAP<sup>Cre</sup>/TG2<sup>Flox</sup> transgenic mouse to determine whether decreasing serotonylation in vivo in the mammary gland during lactation improves maternal bone mass.

Tumor Biology
TUMOR BIOLOGY: DIAGNOSTICS, THERAPIES, ENDOCRINE NEOPLASIAS, AND HORMONE DEPENDENT TUMORS

The Roles of Two Insulin Receptor Isoforms in Triple Negative Breast Cancer Growth

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Systemic hyperinsulinemia is believed to be an important factor in the progression of a number of cancers, including breast cancer by activating the insulin receptor (IR) signaling cascade in the tumor cells. The IR is expressed in two isoforms, IR-A and IR-B. IR-B is the full-length isoform, while IR-A is lacking 12 amino acids in the α-subunit due to exon 11 alternative splicing. IR-A is predominantly expressed in cancer tissues, while IR-B is mostly expressed in metabolic tissues. The IR and closely related insulin-like growth factor 1 receptor (IGF-1R) are expressed in different ratios in cancer cells. Compared with estrogen receptor positive breast cancers, triple negative breast cancers (TNBC) frequently have higher ratios of IR to IGF-1R. Hyperinsulinemia is associated with increased prevalence of TNBC in pre-menopausal women. Although new targeted therapies are emerging, among breast cancer subtypes TNBC continues to carry the worst prognosis and therefore developing a greater understanding of the links between IR signaling and TNBC progression is critical. The aim of this study is to understand the role of IR-A and IR-B on proliferation, metastasis and metabolism in breast cancer cells. We stably overexpressed human IR-A (IR-A OE) and IR-B (IR-B OE) in TNBC MDA-MB-231 and murine c-myc/vegf overexpressing Mvt1 cells with lentiviral transduction using pLVX-IRES-puro HIV-1-based expression vectors with cDNA encoding the human IR-A, IR-B and control cDNA sequences. Native murine IR was silenced using lentiviral transduction of shRNA in the Mvt1 cells. Overexpression of IR was confirmed at a protein level by western blot, and RNA isoform expression was confirmed using real time PCR. Cell proliferation assays were performed in DMEM/10% FBS and revealed that MDA-MB-231 cells with IR-A OE cells had 15% higher proliferation rates than 231 IR-B OE cells. We then examined the IR signaling pathways by western blot in DMEM/10% FBS. No differences in phosphorylated or total ERK1/2