Reproductive Endocrinology

FEMALE REPRODUCTION: BASIC MECHANISMS

Understanding the Influence of Endometrial Cancer Risk Factors Using Human Primary Endometrial Organoids

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MON-LB004

It is unclear how endometrial cancer risk factors such as obesity, high serum testosterone, and high serum levels of the endocrine-disrupting compound bisphenol-A (BPA) influence hormone action to promote carcinogenesis. We hypothesized that obesity, high testosterone, and BPA exposure alters the protective progesterone response in the benign endometrium. Primary human benign endometrial organoids, consisting of both epithelial and stromal cells, were exposed to each of these risk factors *in vitro* in the presence of cyclic levels of estradiol, progesterone, and testosterone for 14 days. Progesterone response genes HSD17B2, IGFBP1, PAEP, and PRL were measured by real-time qPCR and IHC. First, to simulate obesity, endometrial organoids were cocultured with increasing numbers of human adipocyte spheroids during the hormone treatment. Real-time qPCR analysis revealed dysregulation of expression of HSD17B2 and IGFBP1 by approximately 20% when cocultured with 30 adipocyte spheroids. In addition, PRL protein levels were significantly lower in the stroma of the endometrial organoids. Second, increasing concentrations of BPA and 3nM testosterone individually or in combination were added to endometrial organoids together with the 14-day menstrual cycle hormones. Treatment with 0.6 ng/mL of BPA decreased expression of HSD17B2, IGFBP1, and PAEP by 50% to 80%. However, this effect was not seen in the context of high testosterone, indicating that there may be crosstalk between these two risk factors. In summary, this study demonstrated that adipocytes, BPA exposure, and high testosterone directly alter progesterone action in benign endometrial organoids, suggesting a diminution of the protective effects of progesterone and an increased risk of endometrial cancer.

Thyroid

NO LONGER A PAIN IN THE NECK — RECENT INSIGHT INTO THYROID GROWTH, DEVELOPMENT, AND PATHOLOGY

Identification of Novel and Rare Receptor Tyrosine Kinase Fusions in Thyroid Fine Needle Aspirates

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OR28-04

Introduction: Receptor tyrosine kinases (RTKs) initiate signaling cascades, including growth and differentiation. Activation can occur through chromosomal rearrangements that lead to gene fusions. RTK fusions are potential targets for small molecule inhibitors to treat advanced cancers. The original Afirma Xpression Atlas (XA) reported 761 selected variants and 130 fusion pairs in Bethesda III/IV Afirma Genomic Sequencing Classifier (GSC) suspicious or Bethesda V/VI nodules. The landscape of additional potentially actionable gene fusions has not been explored in treatment-naïve patients.

Methods: Anonymized RNA-seq data from >37,000 Bethesda III-VI samples were examined with STAR-fusion to determine gene/gene fusions. All samples were examined for *NTRK1, NTRK3, RET, ALK,* and *BRAF* fusions, regardless of fusion partner. Fusions were evaluated for being in-frame, with an intact kinase domain at the 3' end of the fusion pair. Fusion pairs not currently reported by XA and not reported in thyroid TCGA fusion data are denoted "additional". All fusion pairs were searched for in the literature and public fusion databases.

Results: Examining the Veracyte clinical database revealed 7 additional *NTRK1/3* fusions, with 3 *NTRK* fusions observed more than once - *SQSTM1/NTRK3, VIM/NTRK3*, and *EML4/NTRK3*. One of the 7 *NTRK* fusions had not been previously reported. Eight additional *ALK* fusions were identified, with 4 observed more than once- *ITSN2/ALK, PPP1R21/ALK, PDE8B/ALK, NPAT/ALK*. Five of these 8 ALK fusions had not been previously described. Seventeen additional *RET* fusions were identified, with 5 observed recurrently - *KIAA1217/RET, AFAP1L2/RET, ACBD5/RET, SQSTM1/RET*, and *TFG/RET*. Six of the 17 *RET* fusions had not been previously reported. Seventy-two additional *BRAF* fusions were identified, and 58 of them have not been previously reported. Eight of the 72 *BRAF* fusions were observed more than once. Examining >50,000 Afirma samples, *NTRK1, NTRK3, RET, ALK,* or *BRAF* fusions were not identified among the Afirma GSC Benign, and were present in 3.2% of 16,594 Bethesda III/IV Afirma GSC Suspicious samples, and 8.0% of 1,692 Bethesda V/VI samples. Correlation with surgical histology is unknown.

Conclusions: By examining a large cohort of patients with an unbiased, whole-transcriptome RNA-seq assay, we identified potentially actionable kinase fusions in thyroid nodules beyond those described in TCGA. All fusions described here are either novel and not previously reported, rarely reported in one or two case studies, or not described in thyroid cancers. Additional *NTRK, ALK, RET* and *BRAF* fusions were found, all of which may be targeted with specific kinase inhibitors currently available. Future studies may determine genotype-phenotype correlations regarding the natural history of these neoplasms. Because of the potential clinical implications of these genomic markers for patient management, all 104 fusions described here are now included among the 235 gene pairs reported by the expanded Afirma XA.