

regulated synthesis of target proteins was analyzed using autoradiography and Western blot techniques. Platelets from patients with HIV+ were examined in parallel. RESULTS/ANTICIPATED RESULTS: We identified that highly purified, isolated platelets from healthy subjects possess eRT activity. eRT activity was blocked with the non-nucleoside RT inhibitor nevirapine at concentrations within the therapeutic drug range. LI elements are bicistronic, containing 2 open reading frames (ORFs), ORF1 and ORF2. Thus, we next identified that human platelets express full-length LI mRNA containing ORF1 and ORF2. In human platelets, eRT activity was localized to LI protein containing ribonucleo particles. Platelet eRT reverse transcribed exogenous RNAs, a process inhibited by nevirapine, acting in trans using the 3'-UTR of exogenous mRNAs as a template. To dissect the function of eRT in platelets, we next examined cytoskeletal and protein synthetic events in the presence or absence of nevirapine. Inhibition of eRT in isolated platelets led to characteristically beaded platelets in appearance, strongly resembling bone marrow proplatelets. Parallel increases in platelet reactivity were also observed. As these changes occurred over hours, not minutes, we hypothesized that inhibition of eRT would affect platelet protein synthetic events. Consistent with this, RT inhibition resulted in upregulation of global platelet protein synthesis. We validated upregulation of the synthesis of specific proteins (mitofilin, p-selectin, and L26—a component of the 60S ribosomal subunit essential for mRNA translation). RNA-DNA hybrids, noncanonical nucleic acid structures that regulate gene expression, are enriched in regions where LI is abundant. RNA-DNA hybrids were present in platelets and expression confirmed via differential digestion of RNAs (eg, with RNase A and RNase I). Next-generation sequencing of pulled down (eg, immunoprecipitated) platelet RNA-DNA hybrids identified numerous differentially expressed transcripts and we focused on MAP1LC3B (LC3B), a primary regulator of autophagy. Hybrid sequencing results for LC3B were validated using qPCR and we confirmed that LC3B RNA binds to LI-encoded RNA binding protein. Platelets treated with nevirapine had increased total LC3B protein expression. As RT inhibition is an important mechanism to control HIV infection, we examined platelet morphology, activation, and LC3B expression in platelets from HIV+ subjects treated with nevirapine. HIV+ patients treated with RT inhibitors had higher numbers of platelets that were beaded in appearance at baseline, increased platelet reactivity, and differential LC3B expression compared with healthy controls. DISCUSSION/SIGNIFICANCE OF IMPACT: Taken together, these results demonstrate that platelets possess eRT activity that regulates platelet morphology, platelet hyperreactivity, and protein synthetic events. We postulate that eRT activity in platelets may be a new post-transcriptional regulatory checkpoint. Moreover, our findings have implications in HIV+ patients treated with RT inhibitors, where off-target effects may contribute to platelet activation and an increased risk of thrombosis.

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The role of estrogen receptor- β in the development of the early endometriotic lesion

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OBJECTIVES/SPECIFIC AIMS: To further elucidate the role of estrogen receptor β (ER- β) in the early endometriotic lesion attachment. METHODS/STUDY POPULATION: EECs were immortalized using a telomerase vector. Immortalized cells and parental cells were characterized by genotyping, and expression of ER- β as well as other epithelial cell markers. ER- β was knocked-down in immortalized EECs using lentivirus-mediated shRNA transduction. ER- β knockdown was confirmed by RT-qPCR and Western analysis. EEC cells with or without ER- β knockdown were used to assess their attachment to PMCs in an established *in vitro* assay (Lucidi, 2005). Results were analyzed with Student *t*-test. RESULTS/ANTICIPATED RESULTS: Genotyping using karyotype assay confirmed a normal chromosomal profile. Also positive staining for cytokeratin and lack of any staining with vimentin confirms the epithelial origin of these cells. ER- β knockdown has a significant decrease in attachment compared to control ($p = 0.02$). DISCUSSION/SIGNIFICANCE OF IMPACT: Primary and immortalized cells were 46XX, cytokeratin positive, and vimentin negative confirming their epithelial origin. ER- β knockdown has a significant decrease in attachment compared with control.

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Gut microbiome alterations in children undergoing hematopoietic stem cell transplantation

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OBJECTIVES/SPECIFIC AIMS: Aim 1: To compare microbiome diversity among patients who develop BSI post hematopoietic stem cell transplantation

(HSCT) and patients without BSI. Aim 2: To compare microbiome diversity among patients who develop moderate to severe acute GVHD post HSCT and patients without GVHD. Aim 3: To describe alterations in specific bacterial orders (Enterobacteriaceae, Clostridia, and Lactobacillales) in pediatric patients undergoing HSCT. METHODS/STUDY POPULATION: Next-generation sequencing of the hypervariable V3 region of the 16S rRNA gene isolated from stool specimens collected at baseline (start of preparative regimen to transplant day), early (day 7–14 post HSCT), and late (day 21–28 post HSCT) from 46 children was performed. Microbiome diversity was assessed by the Shannon index as well as UniFrac analysis, and compared among patients with and without GVHD/BSI. Baseline bacterial diversity was compared among patients by primary diagnosis, race, timing of antibiotic introduction and method of supplemental nutrition. RESULTS/ANTICIPATED RESULTS: Median age was 9 years (range 0.5–19.2 years). There were 36 patients with hematologic malignancies. Patients with nonmalignant disease had a characteristic pattern of microbiome diversity (and microbiota order distribution) at baseline that persisted throughout the first month of transplant ($p = 0.004$). Over all patients, there was an early and persistent drop in microbiome diversity throughout the transplant course. Early introduction of broad spectrum antibiotics (prior to transplant day) negatively impacted microbiome diversity ($p = 0.02$). There was no difference in microbiome diversity among patients who developed BSI, when compared with patients without BSI. Similarly, we did not find an association between microbiome diversity and the development of moderate to severe acute GVHD. Ongoing analysis is examining the individual variations in microbiome at the species level, and their relation to transplant characteristics and clinical outcomes. DISCUSSION/SIGNIFICANCE OF IMPACT: In our analysis, microbiome diversity decreased during HSCT, but in contrast to published data, mainly in adult HSCT populations, we found no association between gut microbiome diversity and GVHD or BSI. There are ongoing clinical trials in children and adults using probiotics in HSCT with the aim of decreasing GVHD. Further analysis of our data at the species level may further inform the relationship between gut microbiome alterations and HSCT complications in children and guide clinical interventions.

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PBX1 mRNA expression is a prognostic biomarker of and clinical indicator by RT-qPCR and RNAScope[®] in situ hybridization in neuroblastoma

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OBJECTIVES/SPECIFIC AIMS: (1) Correlate PBX1 mRNA expression as measured by RNAScope in situ hybridization, at an RNA number/cell measurement, Versus by RT-qPCR by the ddCt method. (2) Validate PBX1 mRNA expression in a second independent cohort of neuroblastoma tumor samples, and correlate with patient outcomes. We expect that PBX1 expression will correlate whether detected by RNAScope or by RT-qPCR. This work has the promise of validating a novel biomarker of disease severity, and for clinical translation as the RNAScope technology has been CLIA-certified for clinical use for other genes. METHODS/STUDY POPULATION: Primary neuroblastoma tumor samples were acquired through the Children's Oncology Group Tumor Bank, The Cooperative Human Tissue Network Tumor bank, and the Westmeade Tumor Bank (Westmeade, Australia), with patient outcomes annotated but sequestered until experiments are completed. RT-qPCR is performed using 1 μ g total RNA isolated from each sample by Nucleospin RNA kit (Clontech), reverse transcribed by SuperScript VILO (ThermoFisher Scientific) and amplified using KiCqSTART SYBR Green qPCR mix (Sigma Aldrich). RNAScope was performed on sections of fresh frozen tumor, in triplicate, per manufacturer protocol (ACDBio) using company-designed probes. Statistical analyses performed using GraphPad Prism5. RESULTS/ANTICIPATED RESULTS: PBX1 mRNA expression as measured RNAScope correlated well with matched RT-qPCR values, with most PBX1 transcripts identified within the malignant cells and not in tumor stroma. Correlation with patient outcomes is ongoing (expected to be available at the time of presentation), but as the RNAScope values correlate with $R > 0.9$ with RT-qPCR values, we expect good correlation with outcomes in our primary data set and matching validation set. DISCUSSION/SIGNIFICANCE OF IMPACT: PBX1 mRNA expression is an accurate prognostic biomarker of outcome in low and intermediate-risk neuroblastoma, and testing on an additional validation set is planned based on thresholds established by RNAScope. RNAScope is a method readily translatable to clinical use and its inclusion in future clinical trials will be further studied. It provides an additional benefit that concomitant immunohistochemistry can also be performed. Analysis of high-risk neuroblastomas for responsiveness to retinoic acid based on PBX1 expression is planned.