Introduction: Clinical genome sequencing (GS) promises to incorporate features of multiple prior testing modalities, including identifying copy number variants, single nucleotide variants, mitochondrial DNA variants, and repeat expansions. However, accepted indications for GS remain poorly defined. Moreover, it remains unknown what types of disorders (e.g., redundant testing, clerical errors, better alternative test available, etc.) may occur as this clinical assay becomes more widespread. Methods: Texas Children's Hospital (TCH) initiated its Genetic Testing Stewardship committee in 2021, with representatives from clinical genetics, genetic counseling, clinical molecular genetics, and molecular pathology. With the goal of improving GS utilization at TCH, the committee reviewed the charts of all admitted patients who received GS between March 2020 and May 2022 and extracted data necessary to address the study questions. Results: Seventy admitted patients received GS during the study period. The average age was 4.7 years (two days to 21 years). The majority of patients were in intensive care units (40/70, 57%), especially the general pediatric ICU (23/70, 33%). The most common classes of phenotypes were neurologic or metabolic (43/70, 61%), hematologic or immunologic (9/70, 13%), and cardiac (7/70, 10%). The clinical genetics consult service ordered most GS (66/70, 94%). Nearly half were ordered “rapid” with results on an expedited basis (33/70, average turn-around time of 13.3 days versus 84.6 for non-rapid tests), and all had at least one parent participating. More than half of the tests were characterized as misordered (37/70, 53%), most often due to redundant testing (29/70, 41%). Chromosomal microarray (CMA) was the most frequent prior test (25/70, 36%), though exome sequencing (ES) was also common (19/70, 27%), and patients often had both (13/70, 19%). Patients with prior negative ES had markedly lower diagnostic rates with GS (2/17, 12%) than patients without negative ES (23/48, 48%, OR=0.15, 95% CI 0.015-0.75). The two patients with positive GS but negative ES had final diagnoses likely identifiable by ES reanalysis. Among patients with completed testing, GS had a high diagnostic yield overall (25/65, 38%). However, there were only two cases (3%) in which GS yielded a diagnosis that CMA or ES is unlikely to identify. One patient had a heterozygous 4-Kb single-exon deletion, and the other a mitochondrial DNA variant. Conclusions: Clinical GS is growing in availability, but experience with this testing modality is limited in clinical settings. GS has a high diagnostic yield when used as a first-line test and has the potential to shorten the diagnostic odyssey, but the incremental benefit over other available testing methods may be limited.

Introduction: Loss of BRD7 Promotes Breast Cancer Lung Metastasis by Reprogramming the Tumor Immune Microenvironment

J. Mondal, J. Zhang, F. Giancotti, J. Huse
University of Texas MD Anderson Cancer Center, Houston, TX

Results: In total, 93 rare germline SAI-annotated SVs were identified across 641 unique embedded samples. The aim of this study was to investigate the prevalence of germline SVs in the subset of cancer-relevant genes using tumor-normal whole-exome sequencing (WES) and tumor transcriptome sequencing (WTS). Methods: We established a novel high-throughput in vivo screening platform that enables the identification of specific genetic episthetics that regulate metastatic reactivation. RNA-seq enabled the identification of the core signaling pathways that govern metastatic dormancy, too. Furthermore, tumor space and invasion assays were performed. To decipher the chromatin accessibility regions regulating metastatic reactivation, Chip-seq and ATAC-seq were conducted. A magnetic bead-based multiplex cytokine assay informed us about the regulation of cytokines. In vivo experiments were performed in the 4T07-TGL and D2A1-d dormancy models and the NSG immunodeficient mice model. Further, FACs immune-phenotyping coupled with scRNA-seq was implemented to comprehend the changes in the tumor immune microenvironment upon loss of BRD7. Results: The loss-of-function screen revealed that BRD7 is essential for the maintenance of the dormant state of breast cancer cells in vivo. Interestingly, RNA-seq revealed that BRD7 knockout promotes the expression of genes involved in inflammation, hypoxia, and EMT. Intriguingly, the top signatures enriched in BRD7 silenced cells were IL6-JAK-STAT3 signaling, TNF-alpha signaling, and interferon-gamma responses. Further, ATAC-seq and ChIP-seq experiments indicated that inactivation of BRD7 causes an increased accessibility of enhancer sites that were enriched for interferon-regulated response element sites. Additionally, we found that BRD7 inactivation induces expression of IL6, IL33, CXCL10, and CXCL12, all of which have been implicated in metastasis. Finally, the loss of BRD7 led to upregulation of the tumor-promoting N2 neutrophil population and downregulation of the suppressive M1 macrophages and dendritic cells in vivo. Conclusions: Our novel functional genomc platform shall enable the identification of single genes that enforce dormancy or mediate metastatic reactivation of breast cancer. Furthermore, our findings that BRD7 is a suppressor of breast cancer lung metastasis and a predictive cancer biomarker could have major implications in the formulation of myriad chemotherapeutic strategies for metastatic cancers. Taken together, we anticipate that our study could potentially bring about a paradigm shift in our understanding of how epigenetic regulators, like BRD7, mechanistically regulate breast cancer metastasis and reactivation.

Introduction: Assessment of Germline Splice Variants in Tumor Sequencing

E. Bogdanova1, Z. Soens2, A. Sikov, R. Chen, L. Edelman, B. Funke2, H. Al-Kateb2, F. Hantash2, M. Rossi2
1University School of Medicine at Mount Sinai, New York, NY; 2Sema4, Northbridge, MA; 3Semantics Inc, Framingham, CT

Introduction: Detection and interpretation of splice variants (SVs) that lie beyond the canonical splice acceptor or donor sites can be challenging. We previously evaluated the utility of spliceAI (SAI) in reporting cryptic and/or canonical splice variants in the context of tumor-only sequencing in 2,901 formalin-fixed, paraffin-embedded samples. The aim of this study was to investigate the prevalence of germline SVs in the subset of cancer-relevant genes using tumor-normal whole-exome sequencing (WES) and tumor transcriptome sequencing (WTS). Methods: We chose 641 matched tumor-normal samples across 64 different tumor types which underwent clinical WES/WTS. Rare germline SVs, restricted to 107 genes associated with known and possible increased risk for cancers, were surveyed for potential splicing impact by SAI and RNA analysis. Results: In total, 93 rare germline SAI-annotated SVs were identified across 641 unique samples and were subjected to RNA splicing analysis. A normal splicing pattern was observed for 49.6% (46/93) of annotated SVs. An abnormal splicing pattern was observed for 23.7% (22/93) SVs with the highest proportion on the extended canonical splice-site region. These included: (eight) canonical SVs, (11) non-canonical intronic or cryptic variants, and (three) deep-exonic cryptic SVs. We were unable to determine the splicing pattern for 26.9% (25/93) SVs due to the poor quality of RNA (two), lack of coverage in tumor RNA (15), or absence of the variant in the tumor RNA (eight). SVs with evidence of mis-splicing were identified in known cancer-predisposition genes (ATM, BRCA1/2, MAX, NFKB1, RAD52) and candidate cancer-susceptibility genes (ERCC2, ERCC4, NTHL1, FANCA, FANC, FANCL, FANCJ). ClinVar entries were available for 81.8% (182/22) of RNA-supported SVs with (eight) pathogenic/LP, (seven) variants of uncertain significance, and (three) BENIGH/LB. Somatic loss of heterozygosity (LOH) accompanied 31.8% (72/22) of SVs. Conclusions: Germline SVs in HC susceptibility genes, leading to mis-splicing, were detected in 3.4% (22/641) of WES/WTS specimens in our study. A higher rate of abnormal splicing was noted for non-canonical intronic or cryptic variants (63.6%) versus canonical donor or acceptor sites (36.4%). Using tumor-normal WES and tumor WTS as a standard clinical somatic sequencing approach may assist in reclassification of uncharacterized splicing variants in the known cancer-predisposition genes and extend the spectrum of causative variants in candidate cancer-susceptibility genes. In the somatic context, aberrant germline SVs along with somatic LOH likely contribute to tumorigenesis. However, the significance of some SVs in candidate cancer-susceptibility genes in the germline context remains uncertain and requires further investigation.