ST22. Genome-Wide Copy Number Variation and Targeted Next-Generation Sequencing Studies of Merkel Cell Carcinoma

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Introduction: Merkel cell carcinoma (MCC), an aggressive primary cutaneous neuroendocrine tumor, arises usually due to clonal integration of the Merkel cell polyomavirus (MCPyV) in neoplastic cells and less commonly due to DNA damage by ultraviolet (UV) light. Genetic studies on these MCPyV-positive (MCPyV+) and negative (MCPyV-) subsets of the tumor have shown inter-group differences in the overall mutational burdens and in the mutational profiles of certain tumor suppressor genes (eg *RB1 and TP53).* Our goal was to further investigate genetic patterns in a series of MCCs stratified by viral status. **Methods:** Formalin fixed paraffin-embedded (FFPE) tissue from a cohort of MCCs was used for the study. Twenty-eight cases (9 MCPyV+ and 19 MCPyV-) were analyzed by Oncoscan FFPE kit (Affymetrix, Inc.) to study genome-wide copy number variations (CNV). Targeted next-generation sequencing (NGS) studies, using a panel of 9 genes commonly affected in MCC, was performed on 46 MCCs (21 MCPyV+ and 25 MCPyV-) to identify specific mutations. **Results:** Significantly more CNVs and a greater fraction of the genome was changed in MCPyV- tumors relative to MCPyV+ cases ($p<0.01$ for both comparisons). Extra copies of chromosomes 1p and 3q were commonly found in MCPyV- tumors, but never in MCPyV+ cases, while gains in 1q, 5p, 6, and 19 were common in both. Loss of chromosomes 3p, 4, 5q, 8p, and 13p was common in MCPyV-, but not MCPyV+, cases. Copy number loss of *RB1* or an inactivating *RB1* mutation (either or both) was common in MCPyV- tumors (15/19, 79%) but not MCPyV+ cases (2/9, 22%). A similar trend was seen for *TP53*, with several of the MCPyV- tumors (7/19, 37%) showing gene copy number loss or inactivating mutations compared to none in the MCPyV+ group (0/9, 0%). Three focal CNV findings were observed with similar frequency in virus-positive and -negative groups. The first was biallelic loss at 8p11.22 (9/28, 32%), a region encoding ADAM metalloproteinase domains 3A, 5, and 18. The second was high level gain at 1p13.3 (6/28, 21%), corresponding to genes for glutathione-S-transferase mu. The third, and possibly related, finding was focal loss of genes encoding glutathione-S-transferase theta at 22q11.23 (8/28, 29%). **Conclusions:** Our results support the concept that divergent pathways dependent on either MCPyV or UV light are involved in the tumorigenesis of MCC. The former is driven by few viral oncogenic DNA alterations and the latter by abundant UV light-mediated DNA changes. Loss of genes involved in the ADAM metalloproteinase pathway, already known to occur in high-grade gliomas and neuroblastomas, and dysregulation of the glutathione-S-transferase pathway reflect pathogenetic mechanisms common to both subsets of the tumor and are novel findings.

TT68. Cold Fusion: A Rapid RNAseq Paradigm Using Nanopore Sequencing

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Introduction: Molecular results form a cornerstone of diagnosis. In several entities, such as ALK fusion positive lung cancer or acute myelogenous leukemia with recurrent genetic abnormalities, the identification of a fusion oncogene is pivotal in deciding therapeutic regimen or prognosis. In some cases, such as acute promylocytic leukemia, rapid diagnosis and confirmation of a fusion is critically important. General assays, however, require weeks (*e.g.* massively parallel sequencing), while more rapid approaches, such as FISH, are limited in scope (*e.g.* one gene). New sequencing platforms, such as the Oxford Nanopore MinION system, offer the potential for broad identification of fusion events with a dramatic reduction in turnaround time. We sought to develop a rapid assay for fusion oncogene detection ("Cold Fusion") using the MinION, with the goal of a turnaround time of <24 hours. **Methods:** RNA was isolated from frozen cell line pellets and patient samples with known fusion events. Libraries were prepared using a variety of methods, including an adaptation of the anchored multiplex PCR (AMP) fusion assay as well as unbiased whole transcriptome based sequencing. We also employed direct RNA sequencing and sequencing of full length cDNA libraries to attempt identification of fusion events. **Results:** We confidently identified the BCR-ABL fusion oncogene in a specimen prepared using the AMP sequencing system, and demonstrated detection of the first read containing the fusion <10 minutes from initiating sequencing. Approximately 2% of all reads mapped to the BCR-ABL fusion, likely owing to this gene's substantial overexpression. Though error rates reduced the mapping score, available algorithms mapped fusion reads unambiguously to the whole genome. **Conclusions:** Nanopore based sequencing using the MinION system can dramatically decrease turnaround time for sequencing fusion oncogenes. Despite higher error rates, reads generated by Nanopore sequencing are easily mapped and identify the involved exons with high resolution. Optimization of library preparation protocols offers further improvement of turnaround times and may justify deployment for a set of important clinical scenarios.

ST114. Identification of Germline Variants in Tumor Genomic Sequencing Assays: Usefulness of Variant Allele Fraction and Population Variant Databases

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Introduction: Recently, the Association for Molecular Pathology (AMP), in conjunction with the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP), published consensus guidelines to standardize interpretation and reporting of variants identified in tumor sequencing panels. The recommendations included guidance on distinguishing somatic from germline variants in "tumor-only" workflows. Features suggested to support germline origin included a variant allele fraction (VAF) near 50% or 100% and inclusion in population variant databases, such as the Genome Aggregation Database (gnomAD). We evaluated the predictive value of this recommendation by analyzing a cohort of patients with sequencing results available from both a tumor and a normal sample. **Methods:** Variant information was reviewed from a cohort of 1310 patients enrolled in a large next-generation sequencing research program at our institution (LCCC1108/UNCSeq, NCT01457196). As part of this study, over 200 genes were sequenced in both a tumor and normal sample from each patient. Variants were considered germline if identified in the normal sample. Regions with low coverage (< 100X) in either the germline or tumor sample were excluded from analysis. For all variants, VAF was compared in tumor and normal samples. In addition, gnomAD was queried to determine whether identified variants were included at a population frequency ≥ 1%. **Results:** A total of 37 million variants were identified in normal samples in this cohort. The observed VAF of germline variants in both normal and tumor samples showed a bimodal distribution with peaks centered at 50% and 100%, as expected. However, 22% of heterozygous germline variants (VAF in the normal sample between 40-60%) had a tumor VAF outside of the 40-60% range. Notably, this percentage was quite variable between individual tumors and between tumor types. Specifically, germline variants in tumors of the gynecologic tract and nervous system were more likely to fall within the 40-60% VAF range relative to other tumors, possibly due to a large number of non-malignant tumors from these sites in our cohort. When variants were queried against gnomAD, only 30% of all germline variants in our cohort were present in the database at a population frequency ≥ 1%. **Conclusions:** Our observations indicate that there are considerable limitations to using either tumor VAF or existing databases like gnomAD to identify germline variants in "tumor-only" sequencing workflows. While the AMP/ASCO/CAP recommendation are certain to provide helpful guidance to laboratories, reporting should emphasize the inherent limitations of "tumor-only" workflows in accurately distinguishing germline from somatic variants.