AACR Abstract

Category: MCB09 Genomics, sub area: Functional Genomics

Authors: Suzette Farber-Katz, Vickie Hsuan, Jayne Hoo, Sitao Wu, Huy Vuong, Dong Xu, Hsiao-Mei Lu, Phillip Gray, Aaron Elliott, Rachid Karam

Title: Alternative splicing analysis identifies mutation hotspots in hereditary breast and ovarian cancer genes

Abstract:

Genetic testing for hereditary breast and ovarian cancer (HBOC) is becoming increasingly widespread in the era of precision medicine. The implementation of next-generation sequencing (NGS) has resulted in an explosion of genetic data. While the majority of patients receive definitive results, germline unclassified variants with unknown function are regularly detected in thousands of patients. In particular, variants of unknown significance (VUS) in the HBOC susceptibility genes *BRCA1* and *BRCA2* pose a quandary to medical providers and patients because these genes are clinically actionable. A large percentage of VUS in *BRCA1/*2 are predicted to affect splicing. Previous efforts have focused on interrogating splicing VUS using low-throughput and/or imprecise techniques. Therefore, we developed and validated a method for using RT-PCR NGS to accurately and efficiently characterize germline splicing defects.

The ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium recommends capillary electrophoresis and Sanger sequencing of subcloned transcripts as the gold standard to identify the *BRCA1/2* transcripts that are present in patient samples. We followed the recommendations of the ENIGMA consortium and were able to largely replicate their results when we analyzed the cDNA of lymphoblastoid cell lines (LCLs) generated by the kConFab consortium from carriers of *BRCA1* or *BRCA2* variants known to be associated with splicing defects. In addition to LCLs, we analyzed RNA extracted from normal blood controls and normal breast tissue for the entire *BRCA1* gene. We compared our capillary electrophoresis and Sanger sequencing results to our newly developed RT-PCR NGS assay. For this assay, we used RT-PCR NGS coupled to our custom in-house bioinformatics analysis to identify splicing events including exon skipping, novel intron insertion, and alternative 5' donor and 3' acceptor splicing sites.

Capillary electrophoresis allowed us to roughly visualize the various transcripts present in our samples, though sequencing was needed to confidently identify the exact splicing event. Similar to Sanger sequencing, our RT-PCR NGS assay detected all major splicing events. Interestingly, we found that RT-PCR NGS allowed us to visualize more minor splicing events present in the samples due to our high coverage. We sequenced tens of thousands of reads with RT-PCR NGS, as opposed to Sanger sequencing, which was limited to low-throughput sequencing of single colonies containing subcloned RT-PCR products.

In conclusion, RT-PCR NGS is a reliable high-throughput alternative to the gold-standard splicing assays. Our assay will greatly improve the interpretation of splicing VUS detected by clinical genetic tests for *BRCA1/2*.