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46

Application of long-read sequencing in chronic lymphocytic leukemia cases with complex karyotype

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Introduction: Complex karyotype (CK) typically involves various, often extensive numerical and structural chromosomal abnormalities. In chronic lymphocytic leukemia (CLL), it represents an established adverse prognostic marker. Common methods to detect CK include classical cytogenetics and genomic microarray, however, their resolution is limited. We aimed to explore the ability of long-read sequencing for the precise characterization of complex genomic variants in CLL patient samples.
Methods: CK cases were identified and characterized using classical (IL-2/CpG-stimulated chromosomal banding) and molecular (24×Cyte Multicolor FISH, CytoScan HD Array) cytogenomics. For long-read sequencing, high molecular weight DNA was isolated using chloroform-isopropanol extraction, fragmented by needle shearing, and short DNA fragments were eliminated. The sequencing libraries were prepared using the Ligation Sequencing Kit (Oxford Nanopore Technologies) and sequenced on the MinION or PromethION platform. Reads were aligned to the hg38 human genome reference, and breakpoints were identified with the SVIM variant caller.
Results: For 21 patients, we obtained sequencing data providing 10× (MinION; 4 patients), or >20× (PromethION; 17 patients) average coverage of the genome, with N50 15–25 kb. Identified breakpoints were compared with available cytogenomic results of classical karyotyping, mFISH and genomic microarray. The majority of these results were confirmed by long-read sequencing. In addition, we observed breakpoints in both CLL-associated genes and non-recurring genes affecting cell signaling pathways, which may impact biological processes in CLL cells.
Discussion & conclusion: Our study contributes to a better understanding of the structural variants present in complex CLL genomes and their impact on the leukemic cell phenotype. By confirming previously detected breakpoints using long-read sequencing, we demonstrated the reliability of this approach in characterizing selected chromosomal rearrangements. However, challenges remain in identifying breakpoints in highly repetitive regions. To address this, we intend to employ the T2T human reference.
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