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New technologies and multiomics analysis

LONG-READ RNA SEQUENCING FOR INVESTIGATING BORTEZOMIB RESISTANCE MECHANISMS IN MULTIPLE MYELOMA CELL LINES

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Introduction: : Bortezomib (BTZ), a proteasome inhibitor, significantly improved multiple myeloma (MM) treatment; however, drug resistance remains a major challenge, limiting its effectiveness. Understanding the molecular basis of this resistance is crucial for developing new therapies and overcome the resistance issue. Long-read RNA sequencing offers a powerful method to thoroughly characterize the transcriptome, including full-length transcripts and splice variants, potentially revealing previously unknown mechanisms of bortezomib resistance.

Methods: Total RNA was extracted from BTZ-resistant and BTZ-sensitive clones from two MM cell lines (AMO and H929), in three replicates. After appropriate library preparation, long-reads RNAseq was performed using Oxford Nanopore technology, on the PromethION platform. All the quality control analyses were carried out and transcript were annotated, producing an expression matrix. The R package IsoformSwitchAnalyzeR was used to analyze isoform expression and differential isoform usage of the different transcripts among the conditions.

Results: The Bioinformatic analyses carried out on AMO cell line made possible to observe 53 significant switches (FDR<0.05; absolute dIF>0.1) affecting 45 different genes, involving a total of 93 isoforms. Moreover, two of these genes coded for proteasome subunits (PSMA6, PSMB10).

The H929 cell line was affected by 41 significant switches on 37 genes, with 76 isoforms involved. In both cases, the BTZ-resistant clones showed a significant upregulation of the transcript ENST00000570985, coding for a Nonsense Mediated Decay (NMD)-insensitive variant of the PSMB10 gene, suggesting the potential role of non-coding transcripts in the molecular mechanisms of drug resistance. 18 switching genes were shared between both the BTZ-resistant clones of MM cell lines.

The switching genes were further analysed investigating their ontology and their involvement in pathways through pathway enrichment analysis conducted on Reactome. These analyses highlighted several genes coding for histones (H2AC6, H4C15, H2BC4, H2AZ2, H1-2,H2BC4). Two transcriptomic variants referred to the H2AC6 gene were observed, with a significant increase in the NMD-sensitive transcript ENST00000314088 for the resistant cells, in which almost all the expression is dependent on this non-coding variant, suggesting the relative absence of the related protein. Of note, H2AC6 represents an elite gene related hematologic cancer according to Cancer Gene Census (Disease Ontology: DOID:2531)

Conclusions: Based on what observed, long-reads RNAseq represents an important strategy to better understand isoform usage of specific genes, attributing their overall expression to specific transcript variants, paving the way for a better comprehension of molecular mechanisms underlying resistance to therapy. The high proportion of shared switching genes between BTZ-resistant cell lines, suggests a potential mechanism based on this process.