
Circular RNA-mediated alternative splicing in lung cancer: toward functional isoform restoration
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Abstract:

Background: Alternative splicing is extensively remodeled in cancer and can generate protein isoforms with distinct functional properties. Circular RNAs (circRNAs) are increasingly recognized as regulators of RNA metabolism, but the mechanisms by which they influence alternative splicing in lung cancer remain largely unexplored. We aim to determine whether selected circRNAs modulate splice-site choice and thereby alter the balance of functionally relevant protein isoforms. Methods: A549 non-small cell lung cancer cells and MRC-5 lung fibroblasts were used as malignant and non-malignant models. Total RNA was subjected to ribosomal RNA depletion followed by RNase R treatment to enrich circular transcripts. The enriched RNA was reverse-transcribed and processed by SplintR-mediated ligation, rolling circle amplification, T7 endonuclease I treatment, and size selection. The resulting material is being prepared for Oxford Nanopore long-read sequencing to enable circRNA characterization and isoform-resolved transcript analysis. Candidate circRNA-splicing relationships will subsequently be validated by RT-qPCR and functional perturbation assays. Results: A multistep workflow for circRNA enrichment and amplification was successfully implemented in A549 and MRC-5 cells, enabling progression from total RNA to material suitable for downstream isoform-resolved analysis. The initial experiments confirmed the feasibility of sequential rRNA depletion, RNase R treatment, reverse transcription, SplintR-mediated ligation and rolling circle amplification in both cellular models. Optimization efforts identified amplification efficiency and size selection as the main parameters requiring further refinement prior to long-read sequencing. The established workflow provides a methodological basis for subsequent identification and comparison of circRNA-associated alternative splicing events in malignant and nonmalignant lung cells. Conclusion: This ongoing study addresses an incompletely understood layer of post-transcriptional regulation in lung cancer. Defining circRNA-dependent changes in alternative splicing may reveal mechanisms that shift transcript isoform balance and identify RNA-based targets for restoring functionally favorable protein isoforms.