Exploring treatment response-associated transcript isoform usage in triple-negative breast cancer

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Background: Alternative RNA processing substantially expands transcriptomic diversity and may reveal cancer-associated changes that remain undetected by gene-level expression analyses. However, transcript isoform quantification from shortread RNA sequencing remains challenging, and the reproducibility of isoform-level associations across datasets may be affected by technical and biological variability. We investigated alternative transcript usage in triple-negative breast cancer (TNBC) in relation to response to neoadjuvant chemotherapy. Method: Bulk RNA-seq data from pretreatment TNBC biopsies representing different treatment outcomes were analyzed at gene and transcript levels. Differential isoform usage and isoform switching were assessed together with predicted structural and functional consequences of transcript changes. Selected response-associated events were further explored in an independent TNBC patient-derived xenograft (PDX) RNA-seq cohort stratified according to residual cancer burden. Particular attention was given to the consistency of transcript-level patterns across datasets and experimental groups. Results: Transcript-resolved analysis identified multiple differences in isoform usage associated with treatment outcome, including events not evident from gene-level expression alone. Exploration in the independent PDX dataset revealed that some transcript-level patterns showed substantial variability between datasets, while others retained response-associated differences within individual experimental groups. These observations demonstrate the importance of considering dataset structure and technical variability when interpreting isoform-level associations derived from short-read RNA-seq. Conclusion: Transcript-level analysis provides an additional layer of information for investigating heterogeneity of treatment response in TNBC. At the same time, our results highlight the need for careful assessment of the robustness and reproducibility of candidate isoform-switching events across datasets. Integration of independent cohorts with isoformspecific and long-read validation will be important for distinguishing reproducible biological signals from dataset-dependent transcript variation.