Comprehensive Analysis of Exitron Splicing in Human Cancer

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Background: Alternative splicing (AS) of pre-mRNA plays an important role in generating functional diversity of genes and is frequently observed in cancers. The advances of next-generation sequencing technology allowed researchers to characterize AS in a genome-wide scale and identify unconventional splicing mechanisms that deviate from the well-defined rules of splicing. Recently, a new type of AS events termed exitron (exonic intron) was discovered in plant and human genomes. Exitrons comprise protein-coding intron sequences within protein-coding exons and aberrant exitron splicing was found to play an important role in tumorigenesis and disease progression in breast cancer. However, the functional role of exitron for other cancer types is still unknown.

Methods: We developed novel computational approaches for identification and quantification of exitron splicing events and applied them for analyzing a pan-cancer cohort.

Results: we explored exitron splicing events across 33 The Cancer Genome Atlas (TCGA) cancer types from 9,600 patients by reanalyzing RNA sequencing (RNA-Seq) data. We found exitron splicing events were significantly enriched in tumors compared with normal tissues. Remarkably, we have found tumor specific exitron splicing are enriched in known prostate cancer associated genes, such as FOXA1. Additionally, we found high tumor-specific exitron burden was predictor of poor overall survival in multiple cancers. Gene set enrichment analysis (GSEA) on the top vs. bottom quartile patients by exitron load across TCGA cohorts revealed a significant enrichment in cell cycle pathways (mitotic spindle, G2M checkpoint), and metabolic pathways (oxidative phosphorylation). Comparison between tumor-specific exitrons and somatic mutations indicated that their occurrence are mutually exclusive suggesting exitrons could represent independent oncogenic processes. Finally, exitron-derived neoantigen burden was discovered associated with response to immune checkpoint inhibitor therapy in melanoma and clear cell renal cell carcinoma, indicating that exitron-derived neoantigens may be highly immunogenic.

Conclusions: Our study demonstrates that a) Exitron splicing is a relatively unknown class of RNA level alterations that are prevalent and dysregulated in tumors compared with normal tissues; b) Exitrons splicing is a complementary source of protein-coding alterations that impact driver genes in cancers and may drive tumorigenic progress via distinct biological mechanism and pathways and c) Exitron splicing is a source of neoantigen in cancer.

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