Profiling the splicing landscape in solid tumors in a large, real-world dataset

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INTRODUCTION

- Aberrant pre-mRNA splicing is common across cancers.
- Molecular regulation of splicing is an attractive drug target and clinical trials are currently evaluating splice-targeted therapies (STTs).
- Due in part to the complex and pleiotropic nature of splicing regulation, molecular biomarkers to identify patients who may benefit from STTs are lacking.
- While mutations in splicing factors (SFs) are promising biomarkers, these are rare, leaving many patients with unmet need.
- In this study, we identified and characterized splicing patterns (SPs) in a large, multi-cancer cohort.

METHODS

- De-identified patient records with splicing data from the Tempus xR whole transcriptome assay were extracted from the Tempus clinicogenomic database (N=2918).
- A total of 1052 alternative splicing events were detected in at least 90% of the samples with at least 10 supporting reads.
- Leiden clustering was applied to the percent spliced-in (PSI) values of these events to identify SPs.
- Fisher's exact test was used to assess clinical and molecular enrichment in SPs, and Cox proportional-hazards models were used to assess associations with rwOS and derive hazard ratios (HRs).

Deriving splicing clusters from PSI values

PSI matrix	event 1	event 2	...
Sample 1	0.8	0.01	...
Sample 2	0.1	0.06	...
...	...	...	...

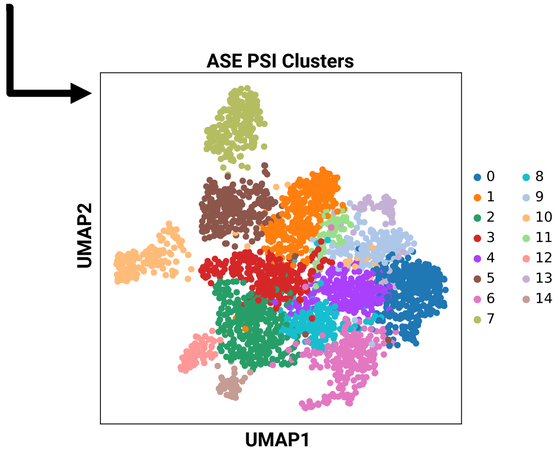

Figure 1. PSI values were used to assign samples to one of 14 splicing clusters. Each point represents a single sample's UMAP embedding, with colors indicating the cluster assignment for that sample.

SUMMARY

- Mutations in splicing factors (SFs) were rare in our cohort, highlighting the need for additional splice-related biomarkers.
- We have identified 15 pan-cancer splicing patterns characterized by distinct clinical and molecular traits.
- Further work will be able to contextualize STT response data using SPs to facilitate STT biomarker discovery.

RESULTS

Splice-derived clusters have distinct clinical and molecular characteristics

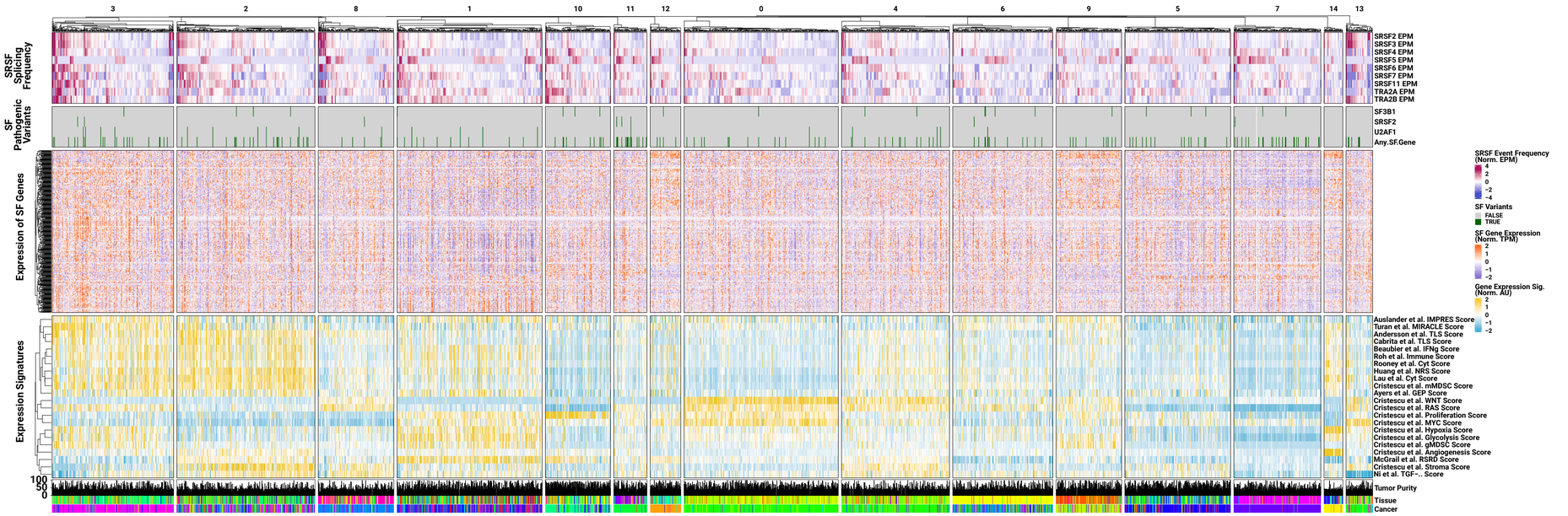

Figure 2. Clinical and molecular features from the Tempus multimodal database were overlaid on the clusters derived from splicing event PSI. Each column in the heatmap represents data from a single sample, and vertical facets represent the PSI-derived clusters shown in Figure 1. Features include: frequency of splicing events in *SRSF* genes represented as events per million mapped reads (EPM); mutations in splicing factor (SF) genes; expression of SF genes; gene expression signatures; tumor purity, biopsy tissue site, and cancer type. Although none of the features displayed in the heatmap were used to derive samples' cluster identities, clear patterns and differences can be observed between clusters.

Frequency of mutations in splicing factor genes by cluster

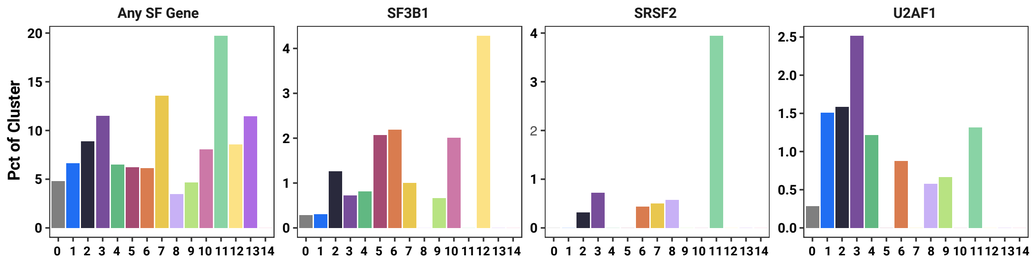

Figure 3. From a list of 404 SF genes 7.7% of patients had one or more pathogenic or likely pathogenic (P/LP) SF variants, and 2.2% had variants in the key biomarker SF genes *SF3B1*, *SRSF2*, or *U2AF1*. Cluster 11 had a significant enrichment of SF variants (19.7% of samples in cluster, P < 0.0005).

Cluster associations with overall survival

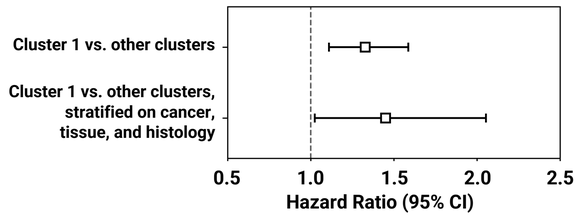

Figure 4. Cluster 1 was enriched for squamous histologies (66.6% of SP, P < 0.0005) and associated with shorter rwOS (HR = 1.33, 95% CI [1.11, 1.59], P < 0.0005; top row). This association remained after controlling for squamous histology (HR = 1.45, 95% CI [1.02, 2.05], P = 0.04; bottom row).

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