Transcriptome analysis of TCGA prostate cancer samples identifies an association of poorer survival and aggressive disease biology with CDC-like kinase (CLK) expression and spliceosome regulation

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Background

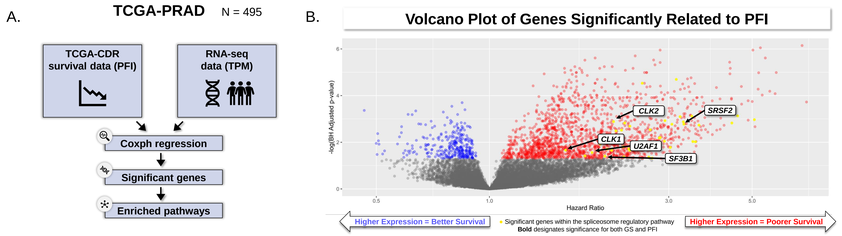
- Alternative splice variants and genes associated with spliceosome regulatory activity show strong correlation with androgen deprivation therapy resistance, tumor progression, and poorer clinical outcome[1–3]
- Pharmacologic targeting of spliceosome-regulating proteins represents a novel treatment approach for prostate adenocarcinoma (PRAD)
- CLKs regulate the activity of serine/arginine-rich splicing factors (SRSFs) that modulate spliceosome assembly, mRNA splicing, and gene expression[4,5]
- This study assessed the association of CLKs and mRNA splicing pathways with clinical outcomes in patients with PRAD from The Cancer Genome Atlas (TCGA) utilizing three metrics: Progression-Free Interval (PFI), Gleason Score (GS), and *PTEN* status

Conclusions

- **Transcriptome analysis of TCGA prostate cancer samples revealed an association of spliceosome activity and *CLK1* and *CLK2* expression with aggressive disease biology in prostate cancer**
- **A Phase 1 study of SM08502, a novel, small-molecule pan-CLK inhibitor, in subjects with advanced solid tumors is ongoing (NCT03355066)**
- **This analysis nominates prostate cancer as a tumor type worth further exploring for the clinical activity of SM08502**

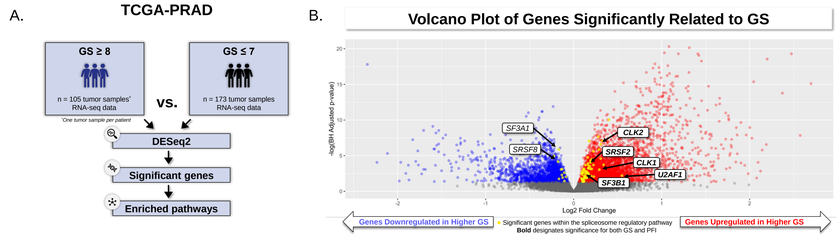
Results

Figure 1. CLK and spliceosome complex gene expression are significantly associated with poorer survival outcome



A. Survival outcome analysis workflow using PFI metrics
B. Volcano plot designating genes significantly associated (*P-adj*<0.05) with poorer survival outcome using PFI
Labeled genes represent CLKs and selected spliceosome complex genes
CLK1: *P-adj* = 2.18E-2, HR = 1.59 (95% CI, 1.19–2.13); ***CLK2:*** *P-adj* = 1.20E-3, HR = 2.14 (95% CI, 1.50–3.06); ***SF3B1:*** *P-adj* = 3.96E-2, HR = 2.03 (95% CI, 1.25–3.29);
SRSF2: *P-adj* = 1.67E-3, HR = 3.29 (95% CI, 1.86–5.82); ***U2AF1:*** *P-adj* = 2.75E-2, HR = 1.87 (95% CI, 1.25–2.81)

Figure 3. CLK and spliceosome complex gene expression are significantly associated with higher GS



A. TCGA-PRAD analysis workflow using GS metric
B. Volcano plot designating genes significantly associated (*P-adj*<0.05) with aggressive disease using GS
Labeled genes represent CLKs and selected spliceosome complex genes
CLK1: *P-adj* = 9.07E-4, log2FC = 0.29±0.08; ***CLK2:*** *P-adj* = 8.40E-8, log2FC = 0.324±0.05; ***SF3B1:*** *P-adj* = 2.55E-3, log2FC = 0.12±0.04;
SRSF2: *P-adj* = 2.16E-5, log2FC = 0.16±0.04; ***U2AF1:*** *P-adj* = 6.34E-3, log2FC = 0.548±0.17

Figure 5. mRNA splicing, Wnt signaling, cell cycle, chromatin remodeling, and DNA damage repair pathways are enriched in the *PTEN* LOF cohort

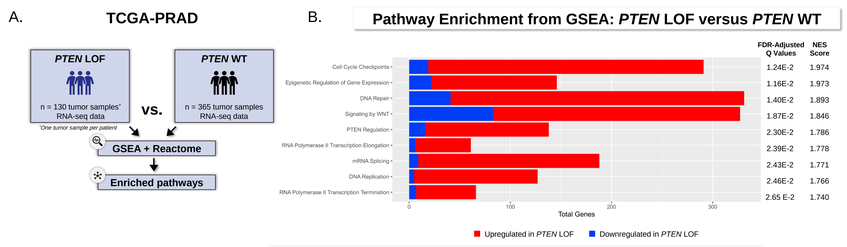


Figure 2. mRNA splicing and cell cycle regulation pathways are associated with poorer PFI

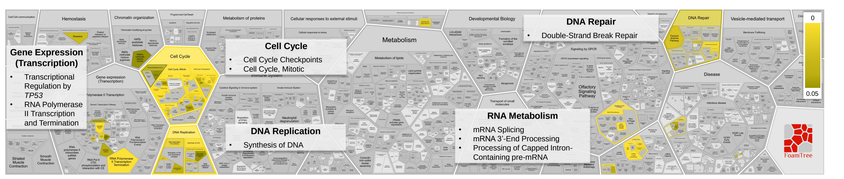


Figure 4. mRNA splicing and cell cycle regulation pathways are associated with higher GS

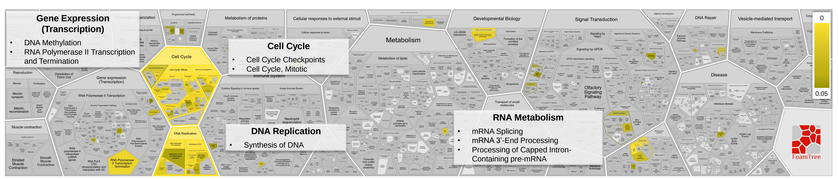
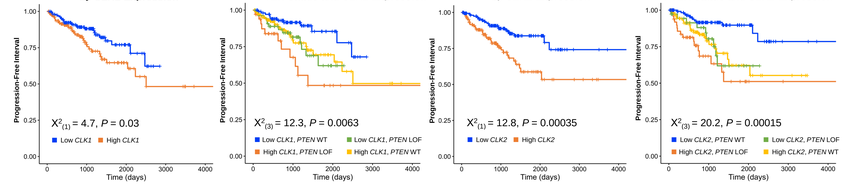


Figure 6. Tumors with low expression of *CLK1* and *CLK2* carried better prognosis than those with high expression



Methods

- Genes significantly associated with PFI were identified using Cox proportional hazards regression models (R v3.6.0, coxph v2.43–3). PFI was obtained through TCGA-CDR,[6] and normalized gene expression values (TPM) were obtained from the UC Santa Cruz Xena Browser. Genes significantly associated with poorer survival were selected with FDR-adjusted Q values <0.05. A subset of CLKs and genes within the spliceosome complex are highlighted within the volcano plot (**Fig. 1**). Genes whose higher expression was significantly associated with poorer survival were run through the Reactome pathway analysis tool[7] to find enriched pathways (**Fig. 2**)
- A similar analysis to the above was run using GS. The PRAD cohort was split into two groups: those with clinical GS sums of ≥8 and those with sums ≤7. Differential gene expression analysis was performed between the two groups with DESeq2 to find significant genes (**Fig. 3**), and Reactome pathway analysis was run to find enriched pathways (**Fig. 4**)
- To further analyze genes and pathways associated with disease aggressiveness, we analyzed *PTEN*, a known mutational biomarker associated with adverse clinical outcomes.[8] TCGA-PRAD was split between *PTEN* loss-of-function (LOF) and *PTEN* wild-type (WT) cohorts, where the former group harbored either deleterious *PTEN* somatic mutations or LOF copy number alterations. Using counts from TCGA, Gene Set Enrichment Analysis (GSEA v3.0, MSigDB Reactome v6.2) was run to find enriched pathways (**Fig. 5**)
- TCGA-PRAD was split into top and bottom tertiles by CLK expression and *PTEN* status. Log-rank tests were used to analyze differences among cohort subtypes, and Kaplan-Meier plots were created to visualize differences in PFI (**Fig. 6**)

References

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