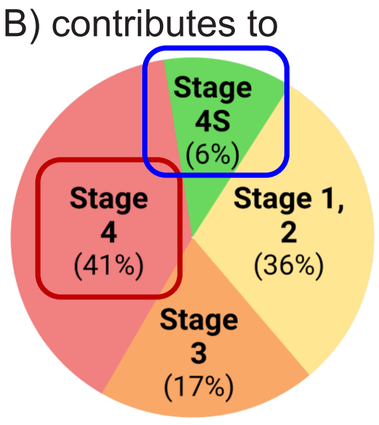
Spontaneous Regression in Neuroblastoma: Analysis of Prognostic LncRNAs

INTRODUCTION

- High clinical variability in neuroblastoma (NB) contributes to variable prognosis [1-4]
- **Stage-specific variability**
  - Aggressive Stage 4 tumors vs. Stage 4S tumors, which may regress [1-5]
- Suggested regression mechanisms [6-7]
  - *NGF*- cell death
  - Anti-tumor immune cell activity



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OBJECTIVES

PURPOSE:
To identify a prognostic lncRNA that may be associated with survival and regression

I. **Identify the mechanisms that differentiate tumors based on survival, and Stage 4 and Stage 4S NB**
II. **Identify a prognostic, good survival, Stage 4S lncRNA**
III. **Elucidate the potential mechanisms of regression based on lncRNA expression**
IV. **Identify the potential role of the lncRNA in NB development**
V. **Analyze the pan-cancer expression of the lncRNA**

METHODOLOGY

Samples
- Unprocessed and uniformly processed NB bulk RNA-seq data (processed data provided by Dr. Modi)
- Pan-cancer bulk RNA-seq data (**Treehouse**)

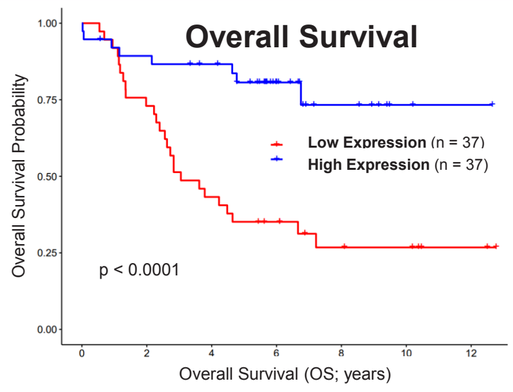
Analyses
I. Differentially Expressed LncRNA – Identification of a high confidence candidate lncRNA using differential expression analysis
II. Prognostic lncRNA – Survival analysis on candidate lncRNA
III. Mechanisms of regression – Associated immune genes and regression genes analysis
IV. Regulation and development – LncMod lncRNA-transcriptional factor-mRNA triplet analysis, NB subtype analysis
V. Pan-cancer expression – Analysis of lncRNA across pediatric and adult cancer

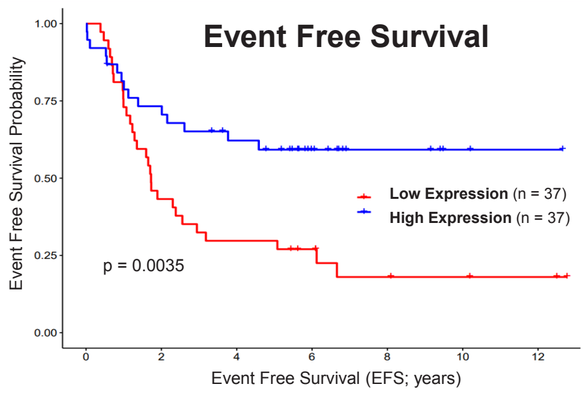
RESULTS/DISCUSSION

I. Identification of a prognostic candidate lncRNA

- ***KLRK1-AS1*** is a high-confidence good survival, Stage 4S antisense lncRNA found on chromosome 12p, in the natural killer (NK) complex [8].

Kaplan-Meier survival





- High expression of *KLRK1-AS1* associated with greater OS and EFS ($p < 0.01$)

Prognostic significance

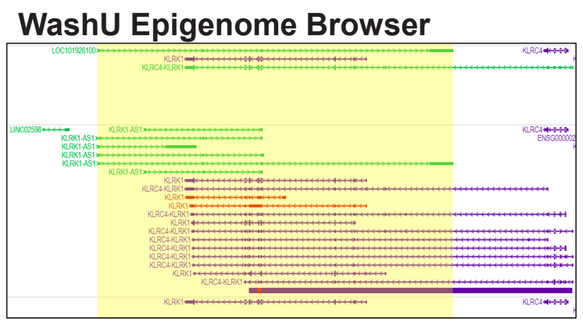
Table 1. Univariate cox proportional hazards.

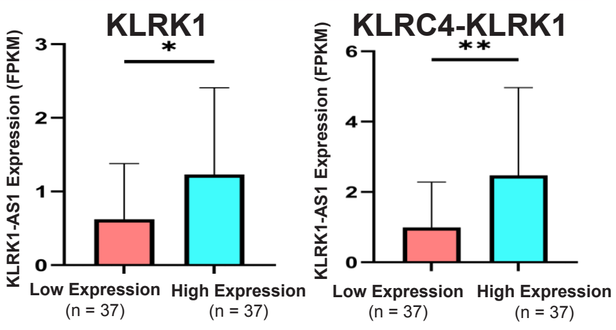
Parameter	Hazard Ratio	95% CI	P-value
Age (> 18 months vs. < 18 months)	0.241	0.0970-0597	0.00214
Risk (High Risk vs. Intermediate, Low Risk)	0.215	0.0783-0.589	0.00281
Stage (Stage 3, 4 vs. Stage 4S)	0.137	0.0335-0.557	0.00549
MYCN Status	0.736	0.484-0.432	0.25700
KLRK1-AS1 Expression (Low Expression vs. High Expression)	0.232	0.105-0.516	0.00033

- *KLRK1-AS1* may have a protective effect on survival ($p < 0.001$; Table 1)

II. Two Mechanisms of Regression

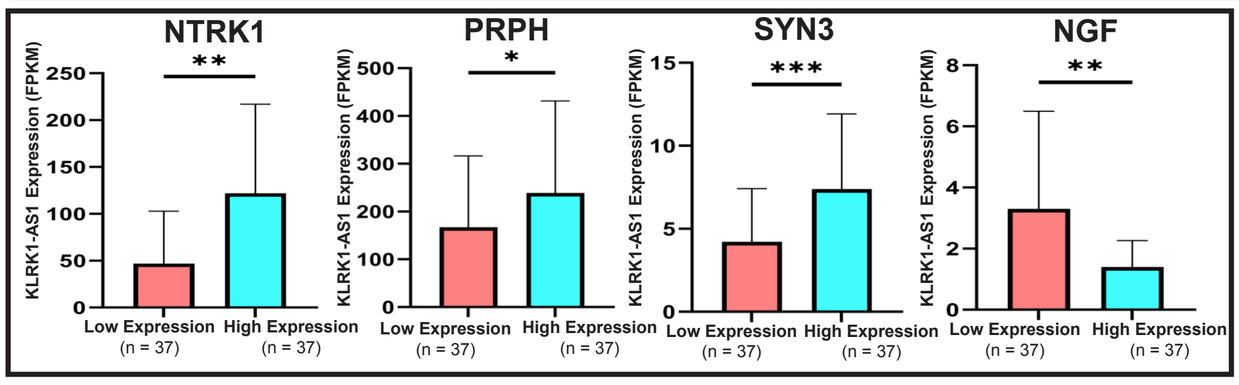
IIA. Immune related genes





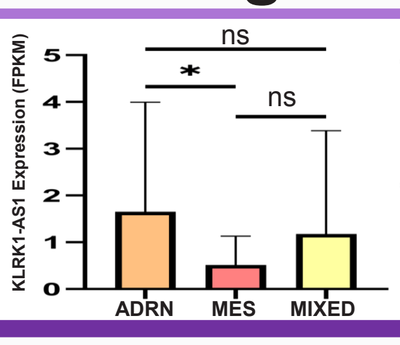
- *KLRK1-AS1* may regulate NK cell activity through its neighboring immune genes [9].

IIB. Regression genes



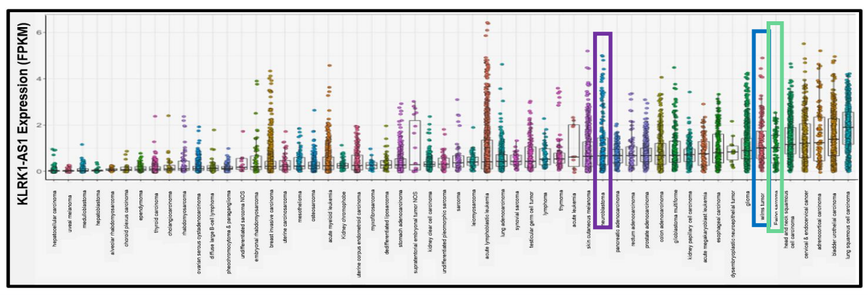
- *KLRK1-AS1* may be associated with differentiation/regression [10].

III. Regulation and development



- *KLRK1-AS1* may interact with core transcriptional circuitry (CRC) members that drive cell state (e.g., *PHOX2B*); [11-13]
- Higher expression of *KLRK1-AS1* was associated with the initially less chemo-resistant ADRN phenotype [14]

IV. Pan-cancer expression



- *KLRK1-AS1* displays variable expression in pediatric cancers, including Wilms Tumor and Ewing Sarcoma.

CONCLUSION: Future Investigations

Phase 2: COMPUTATIONAL
- Multivariate analysis
- Unsupervised clustering
- Bayesian ceRNA network

Phase 3: VALIDATION
- Repeat analysis using larger cohort
- Further assess lncRNA expression in other pediatric cancers

Phase 4: IN VITRO
- Assessment of tumor growth and migration
- Influence on NK activity and cytotoxicity
- Further exploration of cell state