CCN2 and long non-coding RNA AL133346.1 expression are highly correlated in pediatric B-cell acute lymphoblastic leukemia

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INTRODUCTION & AIMS

Acute lymphoblastic leukemia (ALL) is one of the most common childhood cancers. Pediatric B-cell ALL (B-ALL) constitutes a heterogeneous and aggressive neoplasia in which new targeted cancer therapies are required. This subtype involves abnormal proliferation and arrest of differentiation of B cell progenitors.

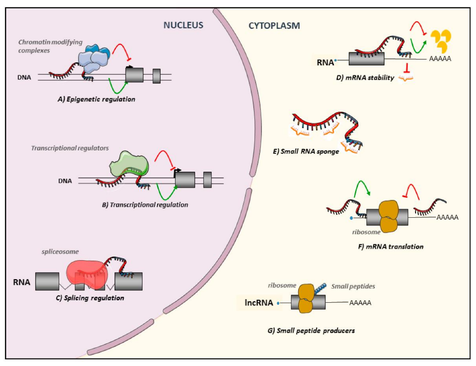


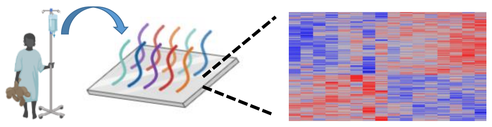
Figure 1 (Gao et al, 2020)

Modifications of tumor-suppressor genes, oncogenic mutations and epigenetic changes have been reported in leukemia. However, a consensus of a clinically relevant lncRNA signature for pediatric B-ALL is yet to be identified.

Long ncRNAs (lncRNAs) are transcripts longer than 200 bp that lack protein coding capacity. LncRNAs are crucial regulators of gene expression due to their involvement in splicing regulation, translation, degradation and stability of mRNAs (Figure 1). that may play an important role in cellular biology.

Dysregulation of lncRNAs has been described in numerous human diseases, including cancer, leading to oncogenic or tumor-suppressive activities. However, few dysregulated lncRNAs have been directly related to leukemia development.

OBJECTIVE → **To identify a signature of differentially expressed lncRNAs and associated protein coding genes in pediatric B-ALL**



PROGNOSIS

DIAGNOSIS

METHODOLOGY

1) Preliminary Analysis: Microarray

42 pediatric B-ALL samples + 4 bone marrow samples

LncPathTM Human Cancer Array
2829 lncRNAs and 1906 protein-coding genes

Gene Expression Analysis
limma package

Hierarchical clustering based on the Spearman correlation

Gene Ontology Analysis
EnrichR software

The differential expression patterns and the strong correlation of AL133346.1/CCN2 in our microarray and another external microarrays led us to further investigate in **TCGA** and **CCLE** databases.

2) External Data Analysis

120 pediatric B-ALL patients + 200 pediatric T-ALL patients

HTSeq Counts – TMM-Normalization
edgeR package

Comparison of gene expression

Survival Analysis (Cox Regression)
survival, *survminer* packages

14 pediatric B-ALL cell lines + 16 pediatric T-ALL cell lines

TPM-normalized gene expression data

Comparison of gene expression

Study of genomic location
GeneHancer database

All analyses and statistical methods were performed using R software

RESULTS

MICROARRAY ANALYSIS

Figure 2 (A) Heatmap of differentially expressed lncRNAs in B-ALL vs. healthy bone marrows: ETV6-RUNX1-negative B-ALL (blue), ETV6-RUNX1-positive B-ALL (red), healthy bone marrows (green). Clustering was performed based on the Spearman correlation coefficient. **(B)** Gene Ontology analysis on the protein-coding genes that were predicted to be functionally associated with the differentially expressed lncRNAs.

Figure 2

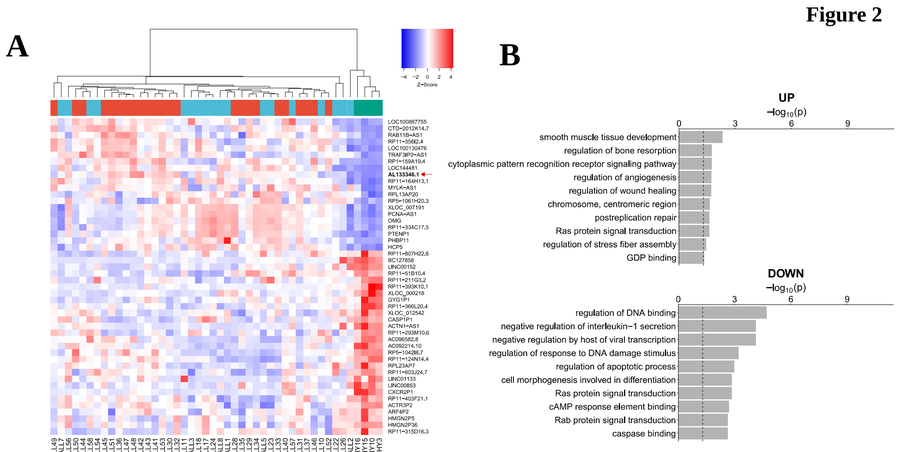
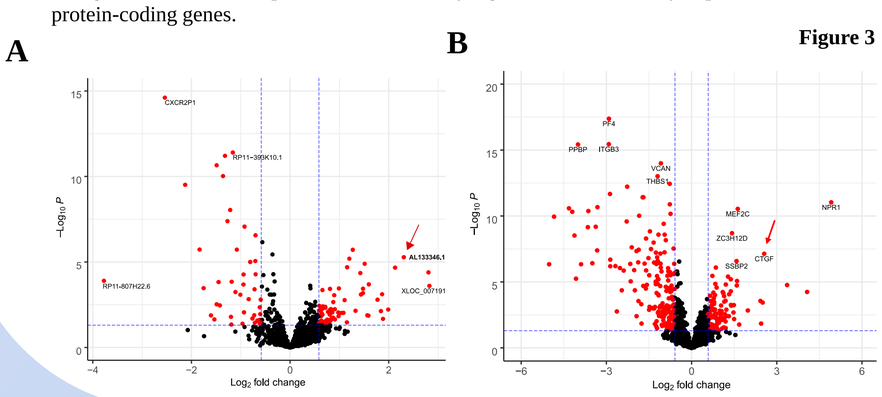


Figure 3. Volcano plot of the differentially expressed lncRNAs **(A)** and protein-coding genes **(B)** in B-ALL vs. healthy bone marrows results. Blue horizontal dashed line represents a threshold of FDR = 0.05. Blue vertical dashed line represents the thresholds of fold change = -1.5 and fold change = 1.5. Red dots represent the statistically significant differentially expressed lncRNAs and protein-coding genes.

Figure 3



EXTERNAL DATA ANALYSIS

Figure 4. Comparison of AL133346.1/CCN2 expression **(A)** and correlation **(B)** between B-ALL and T-ALL pediatric samples from TCGA and CCLE databases. The boxplots and scatterplots are colored according to the ALL subtype.

Figure 4

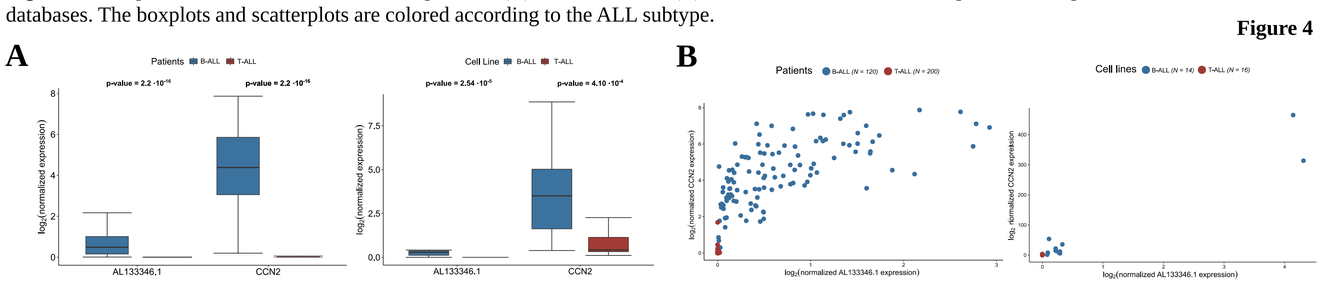


Figure 5. Kaplan-Meier overall survival curves of pediatric B-ALL **(A)** and T-ALL **(B)** patients divided in two groups according to whether AL133346.1 and CCN2 expression was above or beyond the median (B-ALL) or non-null or null (T-ALL).

Figure 5

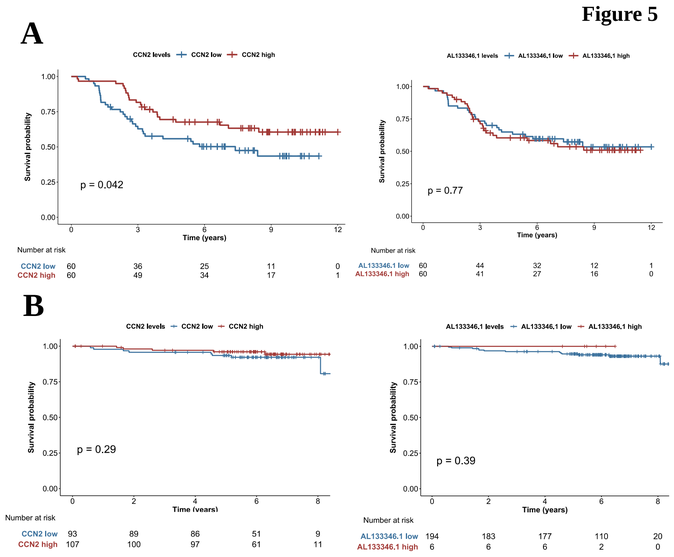


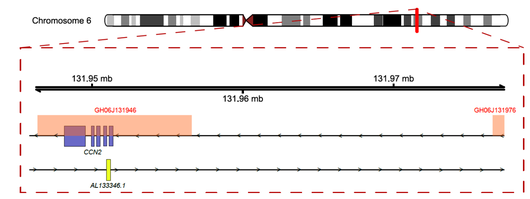
Table 1. Cox Univariate and multivariate overall analysis of clinical covariates in pediatric B-ALL patients from TCGA. clinical Covariates that showed p < 0.2 in univariate analysis were used for a multivariate Cox regression.

Table 1

	Univariate Cox Analysis		Multivariate Cox Analysis	
	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value
Sex (Male vs Female)	1.852 (1.062-3.228)	0.030	1.900 (0.908-3.973)	0.088
Age at Diagnosis (≥ 10 vs 1-9.9 years)	1.500 (0.823-2.733)	0.186	1.140 (0.510-2.550)	0.750
CNS (2-3 vs 1)	1.024 (0.538-1.949)	0.943	NA	NA
t(12;21): ETV6-RUNX1 Fusion (Positive vs Negative)	0.697 (0.250-1.944)	0.490	NA	NA
t(1;19): TCF3-PBX1 Fusion (Positive vs Negative)	21.840 (6.968-68.480)	1.22E-07	7.589 (1.914-30.085)	0.003
t(9;22): BCR-ABL1 Fusion (Positive vs Negative)	0.667 (0.092-4.834)	0.689	NA	NA
Trisomy 4 and 10 - Status (Positive vs Negative)	0.343 (0.192-1.986)	0.418	NA	NA
Ploidy (Hipodiploidy vs Diploidy)	0.126 (0.030-0.531)	0.005	0.237 (0.030-1.881)	0.173
Ploidy (Partial Hyperdiploidy vs Diploidy)	0.383 (0.158-0.926)	0.033	0.650 (0.252-1.679)	0.374
Ploidy (High Hyperdiploidy vs Diploidy)	0.346 (0.143-0.838)	0.019	0.386(0.148-1.006)	0.051
CCN2 Expression (High vs Low)	0.566 (0.328-0.980)	0.042	0.448 (0.204-0.984)	0.045
AL133346.1 Expression (High vs Low)	1.084 (0.632-1.859)	0.770	NA	NA

Figure 6. Genomic location of AL133346.1/CCN2 in chromosome 6. The box with dashed stripes represents a zoom-in of the locus. All CCN2 exons are shown while only the first AL133346.1 exon is included. Shared regulatory regions that could explain AL133346.1/CCN2 coexpression, GH06J131946 and GH06J131976, are represented by red boxes.

Figure 6



CONCLUSIONS

- ❑ **We identified 48 lncRNAs that were differentially expressed between pediatric B-ALL and healthy bone marrow samples.**
- ❑ **B-ALL samples showed a higher expression and correlation levels of AL133346.1/CCN2 than T-ALL samples in external datasets.**
- ❑ **According to the correlation results and genomic location analysis, we propose: (i) AL133346.1 regulates CCN2 expression in cis; or (ii) AL133346.1 and CCN2 are specifically modulated in B-ALL by the same regulatory elements (enhancers).**
- ❑ **Multivariate Cox analysis demonstrated that patients with "high" expression levels of CCN2 showed higher overall survival (OS) than those with "low" levels in pediatric B-ALL patients.**
- ❑ **These results suggest that AL133346.1 could be a possible therapeutic target in the future, and CCN2 could represent an independent prognostic factor in pediatric B-ALL.**

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ACKNOWLEGMENTS

This research has been funded by Aula de Investigación sobre la Leucemia infantil: Héroes contra la Leucemia; the Spanish Ministry of Economy and Business under grant SAF2015-67919-R; the Consejería de Salud de la Junta de Andalucía under grant PI-0245-2017, CS2016-3; the Asociación Española Contra el Cáncer (AECC) Foundation under grant LabAECC-2018; and the Programa Operativo FEDER 2018 under grant B-CTS-126-UGR18.