Testis-associated lncRNAs as therapeutic targets against hepatocellular carcinoma

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ILCA 2020 — 11-13 SEPTEMBER — INTERNATIONAL LIVER CANCER ASSOCIATION — VIRTUAL CONFERENCE

INTRODUCTION

Extensive genetic studies have confirmed the relevance of the aberrant expression of coding and long noncoding RNAs (lncRNAs) for the growth of hepatocellular carcinoma (HCC) [1,2]. This is not surprising since the human genome is populated by genes that express lncRNAs [3]. Many lncRNAs have been described to function in different tumors as drivers of proliferation, metastasis, disease severity, and resistance to treatment. In our lab, we have studied tumor-related lncRNAs using the Cancer Genome Atlas (TCGA) [4] and Genotype-Tissue Expression (GTEx) [5] datasets, which hold remarkably valuable information on gene expression in many tumors and healthy tissues, respectively.

AIM

To identify the healthy tissues that preferentially express lncRNAs upregulated in tumors and validate the potential of these lncRNAs as therapeutic targets against HCC.

METHOD

Using the TCGA and GTEx datasets, we performed pan-cancer and pan-tissue analyses of coding and lncRNAs differentially expressed in tumors and preferentially expressed in healthy tissues or tumors [6]. With these transcripts we performed clinical associations using the TCGA clinical data annotations and an independent cohort of exceptionally well clinically-annotated human samples. Finally, we selected two upregulated candidates from our collection of testis-associated lncRNAs and performed functional analyses to validate their potential as therapeutic targets.

CONCLUSIONS

Some lncRNAs upregulated in hepatocellular carcinoma (HCC) and preferentially expressed in healthy testis are predicted to function as oncogenes and associate significantly with tumor differentiation, tumor burden, and patient survival, suggesting a relevant role in hepatocarcinogenesis and/or tumor evolution. We propose that lncRNAs preferentially expressed in testis are upregulated in cancer and contribute strongly to tumor growth. Targeting these oncogenic lncRNAs should lead to effective therapies with decreased unwanted secondary effects for the treatment of HCC and other tumors.

REFERENCES

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2. Yang, Y. Y. C. T. et al. Recurrently deregulated lncRNAs in hepatocellular carcinoma. Nat. Commun. 8, 1–13 (2017).
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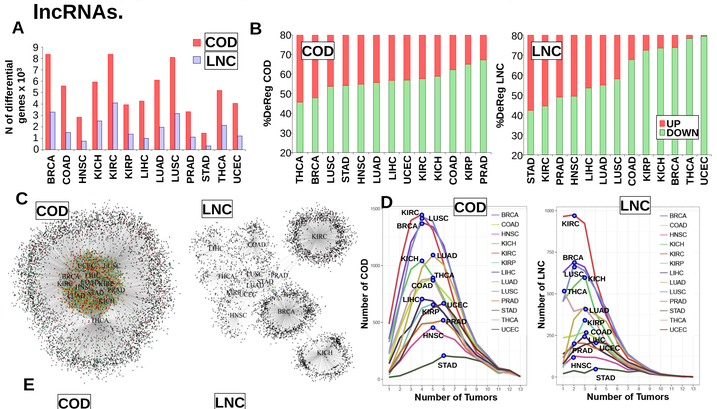
RESULTS

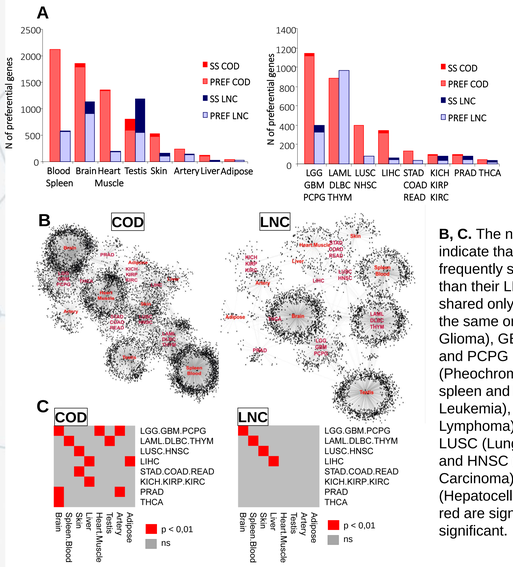
1. Coding genes deregulated in tumors are more promiscuous than lncRNAs.



1. The analysis of coding and non-coding genes in tumors indicates that deregulated coding genes are more promiscuous than non-coding genes. **A**. Coding and non-coding genes deregulated in tumors from TCGA. The characteristics of the analysis and the fact that coding genes are expressed to higher levels than non-coding genes makes the first more abundant. **B**. In most tumors there is a higher proportion of downregulated lncRNAs.

C, D. Deregulated COD genes are very promiscuous, while LNC genes are more tumor-specific. **E.** Heatmap for the enrichment of tumor intersection (–log10 del p value). The analysis highlights the similarities between renal tumors (KIRC, KICH y KIRP) and lung tumors (LUAD y LUSC).

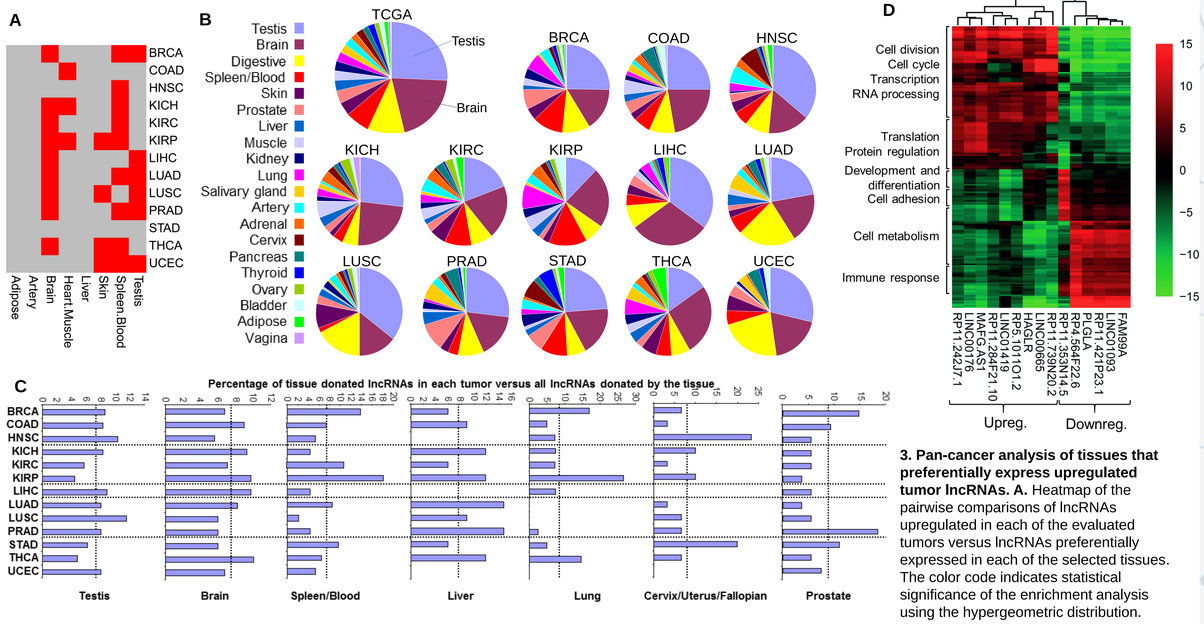
2. Analysis of coding and non-coding genes preferentially expressed in different tumors and tissues



2. Non-coding genes preferentially expressed in tumors and tissues are more specific than coding genes. **A.** We have identified coding genes (COD) and non-coding genes (LNC) expressed preferentially (PREF) or in a superspecific manner (SS) in all tumors from TCGA and all tissues from GTEx.

B, C. The networks and heatmaps (p >0,01) indicate that COD genes are more frequently shared among tissues and tumors than their LNC counterparts. LNC are shared only between tissues and tumors of the same origin: Brain with LGG (Low Grade Glioma), GBM (Glioblastoma Multiforme) and PCPG (Pheochromocytoma/Paraganglioma); spleen and blood with LAML (Acute Myeloid Leukemia), DLBC (Diffuse Large B-Cell Lymphoma) and THYM (thymoma); skin with LUSC (Lung Squamous Cell Carcinoma) and HNSC (Head and Neck Squamous Cell Carcinoma) and liver with LIHC (Hepatocellular carcinoma, HCC). Values in red are significant. Values in grey are non-significant.

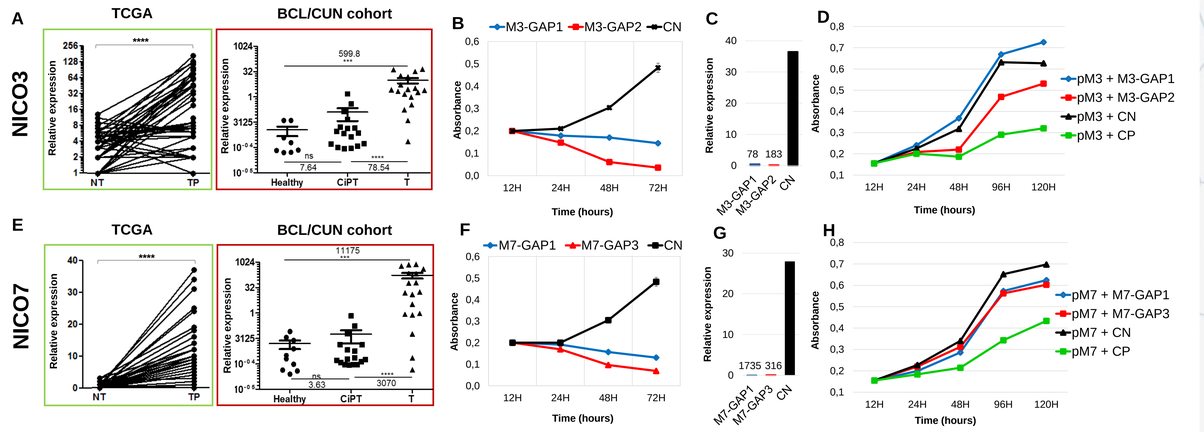
3. Healthy testis and brain preferentially express lncRNAs upregulated in tumors.



3. Pan-cancer analysis of tissues that preferentially express upregulated tumor lncRNAs. A. Heatmap of the pairwise comparisons of lncRNAs upregulated in each of the evaluated tumors versus lncRNAs preferentially expressed in each of the selected tissues. The color code indicates statistical significance of the enrichment analysis using the hypergeometric distribution.

B. Pie chart showing the lncRNA donor tissue of the top 100 upregulated lncRNAs in all (TCGA) or in each of the tumors examined. **C.** Graphs showing the percentage of tissue-donated lncRNAs in each tumor versus all lncRNAs donated by the tissue. The average value is indicated by a discontinuous line. **D.** Gene ontology analysis of the functions enriched for 15 selected lncRNAs. From 1-9 = upregulated. From 10-15= downregulated in HCC

4. NICO3 and NICO7 are overexpressed in HCC human samples and their inhibition impairs HCC cell proliferation *in vitro.*



4. Validation of HCC upregulated lncRNAs in human samples and the effect of their inhibition in HCC cell lines. A, E. Expression levels for the NICOs (**N**on-coding RNAs **I**nduced in **C**ancer with **O**ncogenic features) NICO3 and 7 were measured by qRT-PCR in 45 samples of liver tissue from the BCL/CUN cohort (10 healthy, 17 peritumoral (CiPT) and 18 tumoral (T) samples). Results are shown in charts in red. Charts in green show the expression values of paired samples from the TCGA. Fold increase of expression for each candidate is shown on the top. **B, F.** The expression of NICOs was inhibited in the HCC cell line HuH7 by transfecting two gapmers targeting NICO3 (M3-GAP1 and M3-GAP2) or NICO7 (M7-GAP1 and M7-GAP3) and a non-targeting negative control (CN). Proliferation was measured with MTT assay and inhibition of expression by qRT-PCR (**C, G**).Fold decrease is shown on top of each bar. **D, H.** Co-transfection of plasmids expressing NICO3 (pM3) and NICO7 (pM7) allowed the restoration of transcript levels (as measured by qRT-PCR) (not shown) and the proliferative phenotype of the cells (as measured by MTT). CP, positive control, gapmer against ACTN1 that impairs normal proliferation.