LIN28B Upregulation is Associated with Cancer Stemness and Chemotherapy Resistance in Favorable Histology Wilms Tumor.

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Abstract

Background
Developing chemotherapy resistance in the favorable-histology Wilms tumor (WT) patient-derived xenograft (PDX) KT-47 revealed blastemal enrichment and early, robust upregulation of the *Let-7* microRNA (miRNA) regulator *LIN28B*. We interrogated LIN28B in the original patient (PT-47) after relapse, quantified *Let-7* miRNAs in KT-47, and investigated potential underlying mechanisms of *LIN28B* enrichment.

Methods
Pleural effusion samples were obtained from PT-47 after relapse. Immunohistochemistry (IHC) was performed for LIN28B. In KT-47, miRNA sequencing, whole exome sequencing (WES), 850K MethylationEPIC BeadChip analysis, and single nuclear multiomic transcriptomic/epigenomic profiling were performed. Pearson correlations for *Let-7* levels and validated *Let-7* targets were performed. Relevant genes were overexpressed in a WT cell line and KT-47 spheroids via lentiviral transduction.

Results
Relapsed PT-47 WT cells were positive for LIN28B (Figure 1A). In KT-47, *Let-7* miRNAs were downregulated with chemotherapy resistance (all $P < .05$) (Figure 1B). Reduced *Let-7a* correlated with enriched stemness mediators *HMGA2* ($R^2=0.8665$, $P<.0001$) and *TRIM71* ($R^2=.7381$, $P=.0003$). WES demonstrated no copy number alterations, single nucleotide variants, or insertions/deletions for *LIN28B*, nor was *LIN28B* differentially methylated. Differential methylation correlated with enrichment of embryonic transcription factor *GATA4* (Figure 1D) and stemness-associated multidrug resistance protein *ABCB1* (both $r>0.999$, $P<.001$). Chemotherapy-resistant nuclei demonstrated enhanced chromatin accessibility throughout the *LIN28B* locus (Figure 1E). Nuclei expressing *LIN28B* demonstrated motif enrichment for *GATA4* ($P<.001$), *MYC* ($P<.0001$), and *MYCN* ($P<.05$). Overexpression of NMYC *in vitro* yielded increased protein levels of *LIN28B*, while overexpression of GATA4 decreased LIN28B (Figure 1F). KT-47 spheroids recapitulated protein profiles of KT-47 WTPDX (Figure 1G). LIN28B was successfully overexpressed in KT-47 pretreatment spheroids (Figure 1H).

Conclusions

LIN28B enrichment was confirmed in PT-47 relapse and correlated with a cancer stemness phenotype in KT-47. Enhanced chromatin accessibility and NMYC motif enrichment may drive *LIN28B* transcription. Further *in vitro* and *in vivo* experiments will determine the importance of *LIN28B* in chemotherapy resistance.

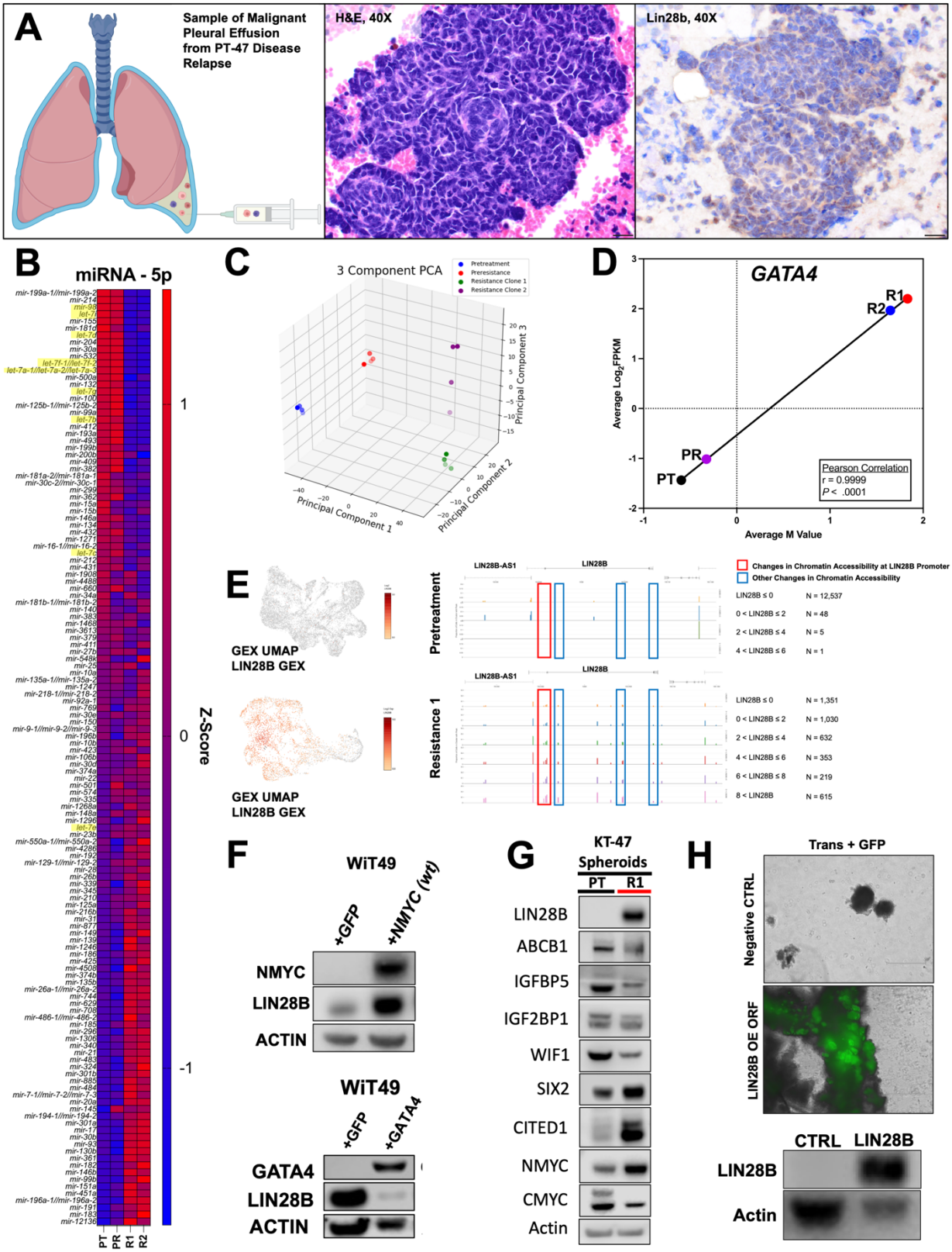
A
Sample of Malignant Pleural Effusion from PT-47 Disease Relapse
H&E, 40X
Lin28b, 40X
B
miRNA - 5p
Z-Score
1
0
-1
PT
PR
R1
R2
C
3 Component PCA
Pretreatment
Preresistance
Resistance Clone 1
Resistance Clone 2
Principal Component 1
Principal Component 2
Principal Component 3
D
GATA4
R1
R2
PR
PT
Average Log2FPKM
Average M Value
Pearson Correlation
r = 0.9999
P < .0001
E
GEX UMAP
LIN28B GEX
LIN28B-AS1
LIN28B
Pretreatment
Resistance 1
Changes in Chromatin Accessibility at LIN28B Promoter
Other Changes in Chromatin Accessibility
LIN28B ≤ 0 N = 12,537
0 < LIN28B ≤ 2 N = 48
2 < LIN28B ≤ 4 N = 5
4 < LIN28B ≤ 6 N = 1
LIN28B ≤ 0 N = 1,351
0 < LIN28B ≤ 2 N = 1,030
2 < LIN28B ≤ 4 N = 632
4 < LIN28B ≤ 6 N = 353
6 < LIN28B ≤ 8 N = 219
8 < LIN28B N = 615
F
WiT49
+GFP
+NMYC (wt)
NMYC
LIN28B
ACTIN
WiT49
+GFP
+GATA4
GATA4
LIN28B
ACTIN
G
KT-47 Spheroids
PT
R1
LIN28B
ABCB1
IGFBP5
IGF2BP1
WIF1
SIX2
CITED1
NMYC
CMYC
Actin
H
Trans + GFP
Negative CTRL
LIN28B OE ORF
CTRL
LIN28B
LIN28B
Actin

Figure 1. (A) Schematic of pleural effusion sampling from PT-47 with representative images demonstrating hematoxylin-and-eosin-staining of relapsed tumor cells and LIN28B immunohistochemistry. **(B)** Heat map of Z-scores demonstrating differential expression of miRNAs with the development of chemotherapy resistance. *Let-7* family members are highlighted in yellow. **(C)** 3 component principal component analysis demonstrating clustering of treatment phenotypes by global DNA methylation status. **(D)** Correlation of DNA methylation with *GATA4* enrichment during the development of chemotherapy resistance in KT-47. **(E)** Gene expression UMAPs demonstrating an increased number of nuclei expressing *LIN28B* at the Resistance 1 phenotype / timepoint compared to the pretreatment timepoint. The *LIN28B* locus in Resistance 1 nuclei demonstrated chromatic accessibility peaks absent in the Pretreatment nuclei. **(F)** Western blots of whole cell lysate from Wilms tumor cell line WiT49 demonstrating upregulation of LIN28B with NMYC over-expression and downregulation of LIN28B with GATA4 overexpression. **(G)** Western blot comparing the protein profiles of KT-47 Pretreatment and Resistance 1 spheroid cultures. **(H)** Light microscopy demonstrating successful lentiviral transduction of KT-47 pretreatment spheroids via human *LIN28B* open reading frame, with protein-level expression confirmed on Western blot. “PT-47” = patient whose tumor contributed to the KT-47 model; “H&E” = hematoxylin-and-eosin; “miRNA-5p” = 5-prime microRNA; “PT” = pretreatment; “PR” = Preresistance; “R1” = resistance clone 1; “R2” = resistance clone 2; “PCA” = principal component analysis; “FPKM” = fragments per kilobase million; “GEX” = gene expression; “UMAP” = uniform manifold approximation and projection; “GFP” = green fluorescent protein; “wt” = wild type; “OE” = overexpression; “ORF” = open reading frame; “CTRL” = control.