microRNA 5p and 3p strands mediate coordinated regulation and recurrently modulate pan-tumor oncogenic pathways

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Abstract

Only one microRNA (miRNA) strand (5p or 3p) was considered functional, but recently both strands have been recognized as active, leaving half of all miRNA uninvestigated[1,2]. To determine whether both miRNA strands, which have different mRNA-targeting sequences, cooperate to regulate core pathways/functions across cancer types, we evaluated genomic, epigenetic, and molecular profiles of >5200 patient samples from 14 different cancers in TCGA, and RNA interference and CRISPR screens in >280 cancer cell lines[3]. We identified dysregulated miRNA 5p/3p pairs that coordinately modulate oncogenic pathways and cell survival/growth across cancers[4]. Two miRNA showed down-regulation of both strands recurrently increased cell cycle pathway genes and significantly reduced patient survival in multiple cancers. Forced expression of both strands showed cooperativity in reducing cell cycle pathways and inhibiting lung cancer cell proliferation and migration. Therefore, we identified miRNA where both strands function together in core processes/pathways essential for tumorigenesis and reveal a previously unknown pan-cancer miRNA signature.

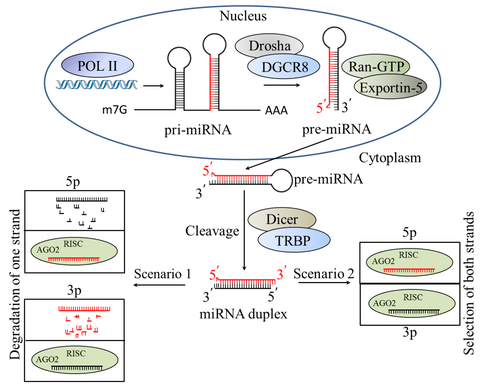


Figure 1. Selection of mature miRNA strand and the fate of its passenger strand.

Methods

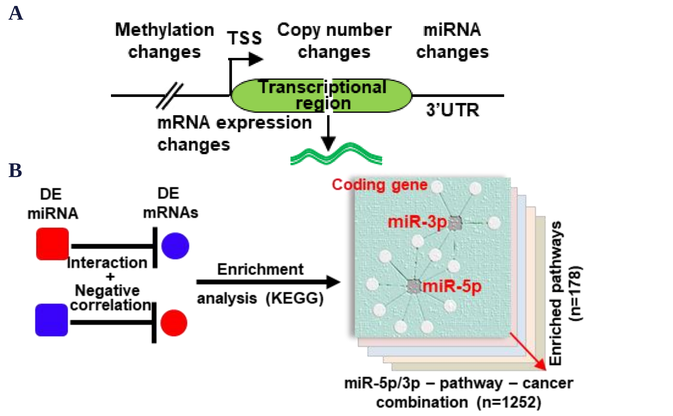


Figure 2. Integrative bioinformatics framework to predict miRNA 5p/3p mediated gene regulation in 14 TCGA solid cancer types.

Results

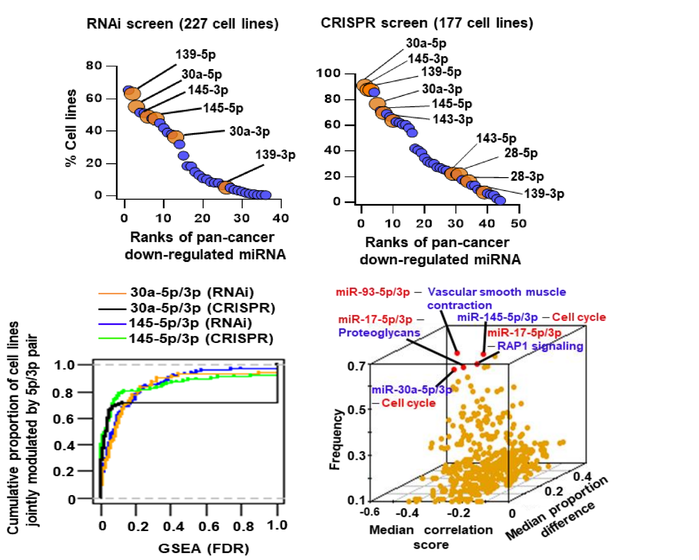


Figure 3. Pan-cancer cell survival/growth modulation (A and B) by miR-30a and miR-145 5p/3p pairs through the regulation of cell cycle pathway (C).

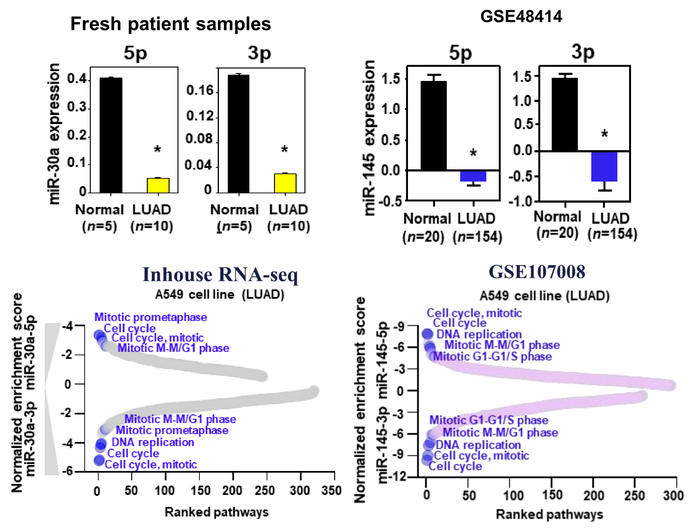


Figure 4. Dysregulation of individual strand of miR-30a and miR-145 (A) independently modulates cell cycle associated pathways (B) in lung adenocarcinoma; *$P < 1.99x10^{-6}$.

Results (cont.)

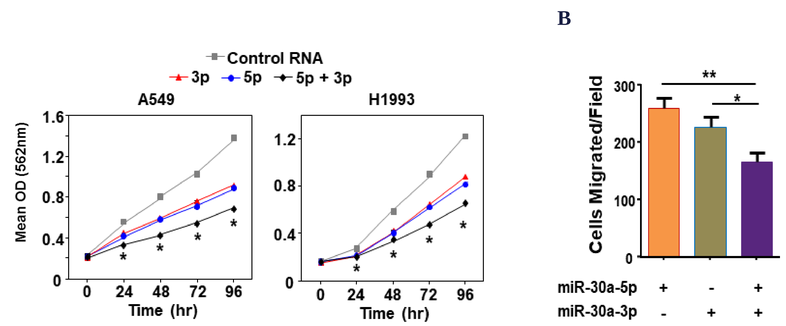
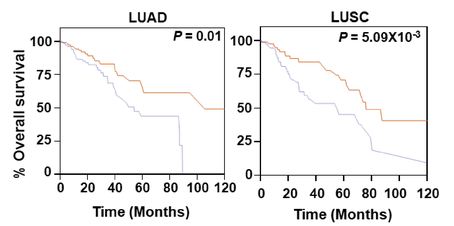


Figure 5. Cooperative effect on lung adenocarcinoma cell proliferation (A) and migration (B) after transfecting single or both strands of miR-30a; *$P < 2.43x10^{-3}$.



Signature miRNA expression

Low — High

Cancer type	Number of samples Low (n)	High (n)	Log-rank P
KIRC	63	64	$6.37X10^{-4}$
LUSC	84	85	$5.09X10^{-3}$
LUAD	110	110	0.01
CHOL	9	9	$7.60X10^{-3}$
BRCA	189	189	0.06

Figure 6. A four-miRNA (both strands of miR-30a and miR-145) expression signature correlates with patient overall survival across diverse TCGA cancer types.

Conclusions

- Several miRNA 5p/3p pairs, which do not have identical mRNA-targeting seed sequences, coordinately regulate the same tumorigenic processes in cancer (reproducible associations between miRNA 5p/3p dysregulation and modulation of cancer cell viability/growth).
- Identification of recurrence of previously unknown miRNA 5p/3p and pathway associations across 14 cancer types.
- Over-expression of two top predicted miRNA, miR-30a and miR-145 5p/3p pairs, reduced lung cancer cell growth and movement, and act as a miRNA signature that has patient prognostic power across multiple cancers.
- Development of a novel integrative bioinformatics approach that can now be used to determine cooperativity of other genes in cancer and other diseases.

References

[1] Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic Acids Res.*, 42: D68-73, 2014.

[2] Misono S, et al. Dual strands of the miR-145 duplex (miR-145-5p and miR-145-3p) regulate oncogenes in lung adenocarcinoma pathogenesis. *J Hum Genet.*,63(10): 1015-1028, 2018.

[3] McFarland JM, et al. Improved estimation of cancer dependencies from large-scale RNAi screens using model-based normalization and data integration. *Nat Commun.* 9(1): 4610, 2018.

[4] Mitra R, et al. Pan-cancer analysis reveals cooperativity of both strands of microRNA that regulate tumorigenesis and patient survival. *Nat Commun.* 11(1):968, 2020.