# OncomiR Passenger Strand Activity?

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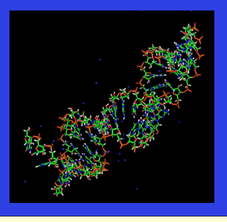


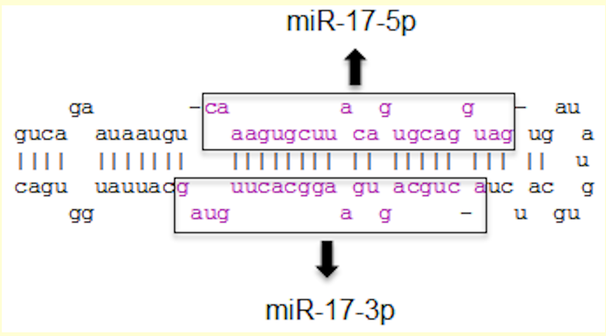
## ABSTRACT

We hypothesized that knocking down the oncogenic microRNA (oncomiR) miR-17-5p might restore the expression levels of programmed cell death 4 (PDCD4) and phosphatase and tensin homolog (PTEN) tumor suppressor proteins, illustrating a route to oligonucleotide therapy of TNBC. Contrary to conventional wisdom, we found that antisense DNA-LNA knockdown of miR-17-5p guide strand reduced PDCD4 and PTEN proteins by 1.8±0.3 fold in human triple negative breast cancer (TNBC) cells, instead of raising them. In contrast, antisense DNA-LNA knockdown of miR-17-3p passenger strand maintained PDCD4 and PTEN protein levels. Furthermore, antisense DNA-LNA knockdown of miR-17-3p passenger strand raised *PDCD4* mRNA by 25±2% and *PTEN* mRNA by 22±6%.

## BACKGROUND

A variety of oncomiRs are overexpressed in TNBC, and are being studied intensively as targets for complementary oligonucleotide therapy.[1] Conventional wisdom holds that only one of the two strands in an oncomiR precursor duplex is selected as the active oncomiR guide strand.[2] The complementary passenger strand, however, is thought to be inactive.[2] High levels of the oncomiR guide strand called miR-17-5p can inhibit ribosomal translation of tumor suppressor gene mRNAs, such as *PDCD4* or *PTEN*.[3] Blocking 3'UTR sites depresses initiation of *PDCD4* or *PTEN* mRNA translation in TNBC cells, interdicting tumor suppression.[4,5]

## miR-17 HAIRPIN

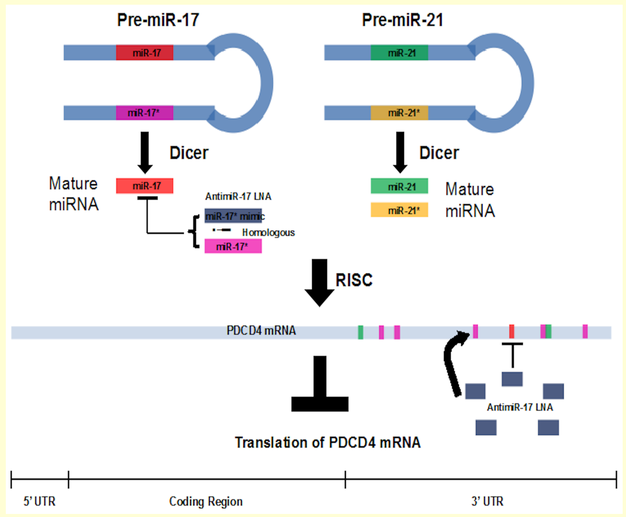



## *IN SILICO* TARGET SEARCHES

We sought an answer to the knockdown contradiction by applying 3 different oncomiR target prediction algorithms, rna22[6], TargetScan[7], and miRanda[8] to search for *PDCD4* and *PTEN* mRNA targets of oncomiRs miR-17 and miR-21. Sequence scanning yielded only one miR-17-5p guide strand site in *PDCD4* or *PTEN* mRNA. OncomiR miR-21-5p can bind two 3'UTR targets in *PDCD4* and a single target in *PTEN* mRNA.

## PASSENGER STRAND SITES

In contrast, 5 putative binding sites for miR-17-3p passenger strand in *PDCD4* mRNA, and 6 in *PTEN* mRNA, were predicted. No sites, however, were identified for the passenger strand miR-21-3p.




**Predicted miR-17-3p passenger strand binding sites in the 3'UTR of *PDCD4* mRNA**

| Site | Algorithm[a] | Mfold Predicted Structure[b] | Mfold[c] ΔG°, kcal/mol |
|---|---|---|---|
| 1682-1703 | rna22 | 5′-UGAA AA- - - UAUAAG C UC UUGCAGU AUGUUC G AG GACGUCA-5′ G--- ACG A U | -12.3 |
| 2009-2030 | rna22 | 5′-CAAGG - -- AC AGUGUUUUU UGUAGU UG UCACGGAAG ACGUCA-5′ GA--- U UG | -14.1 |
| 2800-2821 | rna22 | 5′-C------ AAAUA G C UGCC UU ACUGCAG ACGG AA UGACGUC GAUGUUC ----- G A-5′ | -14.1 |
| 3315-3336 | rna22 | 5′-C G - AGAGG CUG GAG GC UUGCAGU GAU UUC CG GACGUCA-5′ G A GAAGU | -15.2 |
| 1110-1136 | miRanda | 5′-UUUAAAC AAAUA G C UGCC UU ACUGCAG ACGG AA UGACGUC GAUGUUC ----- G A-5′ | -14.1 |

[a]The potential oncomiR:mRNA binding sites were identified by rna22, Targetscan, or miRanda.
[b]Top strand is mRNA (5'→3') and the bottom strand is oncomiR (3'→5').
[c]Calculated at http://mfold.rna.albany.edu/?q=mfold.

**Predicted miR-17-3p passenger strand binding sites in the 3'UTR of *PTEN* mRNA**

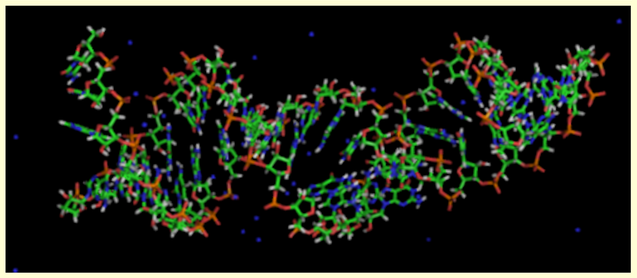
| Site | Algorithm[a] | Mfold Predicted Structure[b] | Mfold[c] ΔG°, kcal/mol |
|---|---|---|---|
| 3768-3789 | rna22 | 5′-CUAU - AA UACAA UG UUU UGCAGU AUGUU AC AAG ACGUCA-5′ G--- C GG UG | -11.9 |
| 7644-7665 | rna22 | A AGA A 5′-ACAG UGC UUAC UGUAGU UGUU ACG AGUG ACGUCA-5′ GA C GA- - | -14.4 |
| 8937-8418 | rna22 | 5′-GGAAUG AG--- U AAGUG GCU GUAGU UUCAC UGA CGUCA-5′ GAUG-- GGAAG | -10.0 |
| 8440-8461 | rna22 | 5′-UGC - G CAAGU CU UUUACUGCAGU GUUCA GG AAGUGACGUCA-5′ GAU C | -19.8 |
| 8477-8498 | rna22 | 5′-UUU U G - C CAG GUG UUUU CUGUAG GUU CAC GAAG GACGUC GAU - G U A-5′ | -11.7 |
| 8628-8649 | rna22 | 5′-CC - U - AAUG CU CA GU CCU UGCAGU GA GU CA GGA ACGUCA-5′ U U C AGUG | -11.1 |

[a]The potential oncomiR:mRNA binding sites were identified by rna22, Targetscan, or miRanda.
[b]Top strand is mRNA (5'→3') and the bottom strand is oncomiR (3'→5').
[c]Calculated at http://mfold.rna.albany.edu/?q=mfold.

## MOLECULAR DYNAMICS

We simulated all predicted 3'-UTR mRNA:oncomiR duplexes, for both guide strands and passenger strands. We calculated 50 ns accelerated molecular dynamics of each duplex in explicit $H_2O$ with 100 mM NaCl with AMBER 12.[9] Our molecular dynamics calculations predicted stable A-form duplexes for all oncomiR passenger strand:mRNA targets, as well as for guide strands.

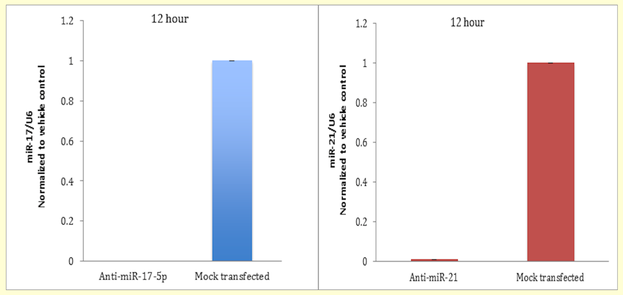
## STRUCTURAL PREDICTIONS



The structural predictions imply that 3'-UTR mRNA:oncomiR duplexes can be accommodated in the substrate groove of Ago2, despite the mismatches and bulges that appear so distorted in the Mfold presentation. Our result agrees with an earlier simulation of an 11mer duplex bound to *Thermus thermophilus* Ago.[10] MMPBSA calculations[9] predicted favorable ΔG° for each duplex formation, as did Mfold.[11]

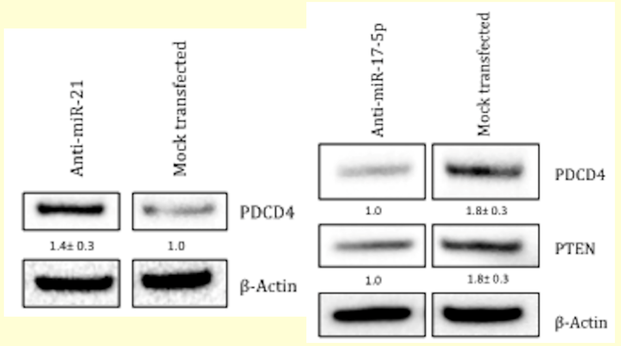
## ONCOMIR KNOCKDOWN

Anti-miR-17-5p DNA-LNA chimera (Exiqon) transfected into MDA-MB-231 TNBC cells knocked down miR-17-5p by 99±0.01% after 12 hr (left). Similarly, anti-miR-21-5p transfected into MDA-MB-231 TNBC cells knocked down miR-17-5p by 99±0.04% (right). The oncomiRs were knocked down.



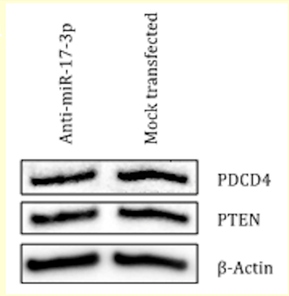

## WESTERN BLOTS

To evaluate whether miR-17-5p had any effect on the expression level of PDCD4 protein, we transfected anti-miR-17-5p into MDA-MB-231 TNBC cells and analyzed protein levels 48 hr after transfection. Surprisingly, the PDCD4 protein and PTEN protein levels were down-regulated by 1.8±0.3 fold, instead of being up-regulated as expected following miR-17-5p knockdown. This result conflicted with the expectation that miR-17-5p target expression would increase when miR-17-5p was knocked down. Anti-miR-21-5p, however, behaved as expected, elevating PDCD4 and PTEN protein levels.




## WESTERN BLOTS

To determine if the passenger strand was involved in the contradictory results above, we knocked down endogenous miR-17-3p with anti-miR-17-3p. The




western blot after miR-17-3p knockdown showed no significant changes in PDCD4 or PTEN protein levels. The static result is plausible, because there are 5-6 potential binding sites for miR-17-3p on *PDCD4* and *PTEN* mRNAs, vs. one for miR-17-5p.

## CONCLUSIONS

We concluded that anti-miR-17-5p guide strand mimicked miR-17-3p passenger strand, effectively raising the miR-17-3p concentration in TNBC cells.

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5′ A-CUGCAGUG-AAGGCAC-UUGUAG 3′  miR-17-3p
5′ ACCTGCACTGTAAG-CACTTTG  3′  Anti-miR-17-5p

5′    CAAAGUGC-UU-ACAGUGCAGGUAG 3′ miR-17-5p
5′ TAC-AAGTGCCTTCAC--TGCAG 3′ Anti-miR-17-3p
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Our results imply that therapeutic antisense sequences against oncomiRs should be designed to target the oncomiR strand with the greatest number of putative binding sites in the target mRNAs**. Precaution must be taken when designing oncomiR knockdown sequences as potential therapeutic or diagnostic agents, since both the guide strand and the passenger strand of a oncomiR might target the same transcript. Both strands act.**

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