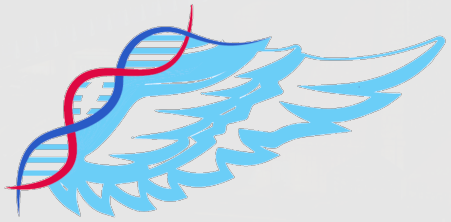
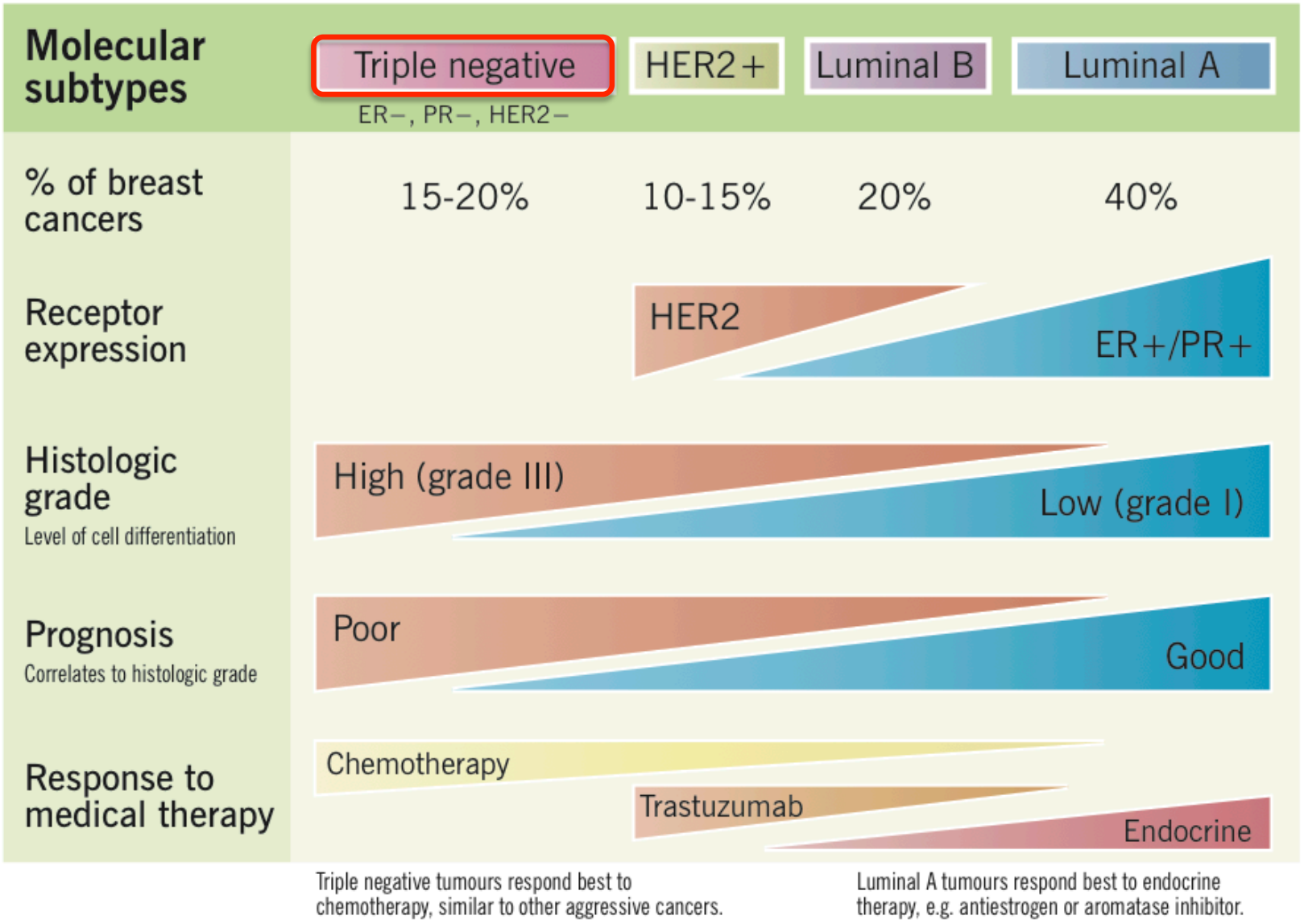
Triple Negative Breast Cancer Therapy by microRNA Blockade with PNA-peptides, without Passenger Strand Side Effects

XXII International Roundtable on Nucleosides, Nucleotides and Nucleic Acids



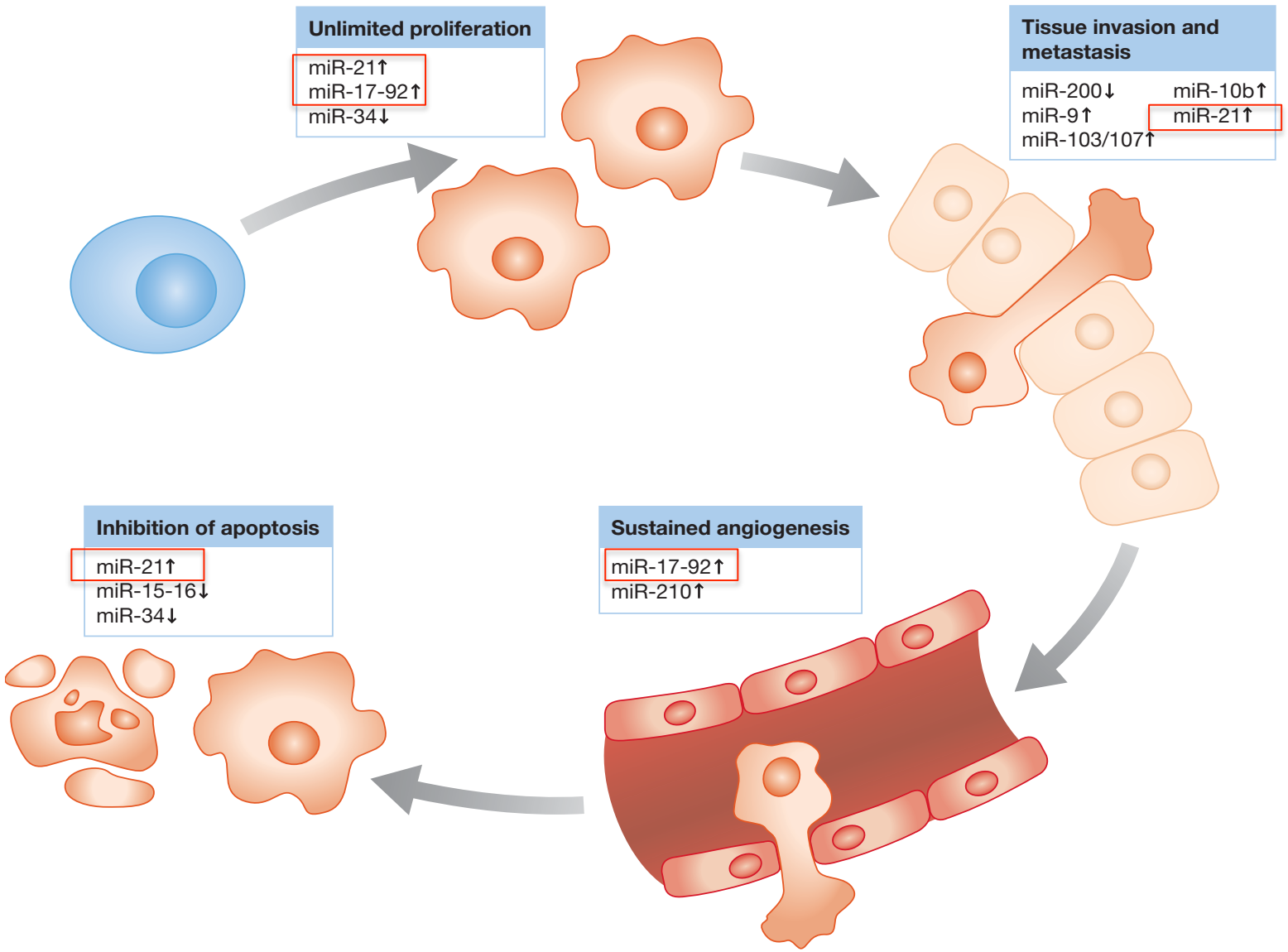
Bound Therapeutics LLC

Breast cancer subtypes



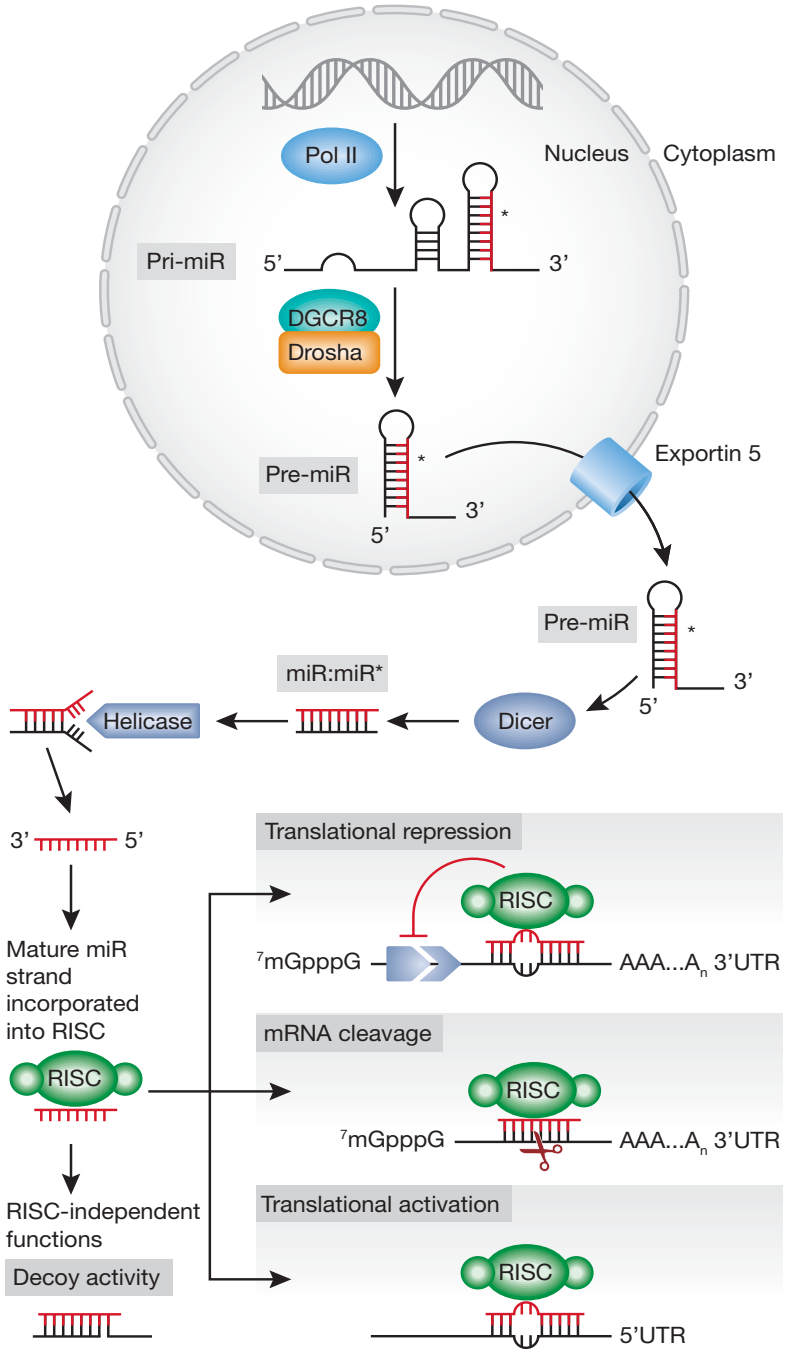
http://www.pathophys.org/breast-cancer/

OncomiRs in cancer



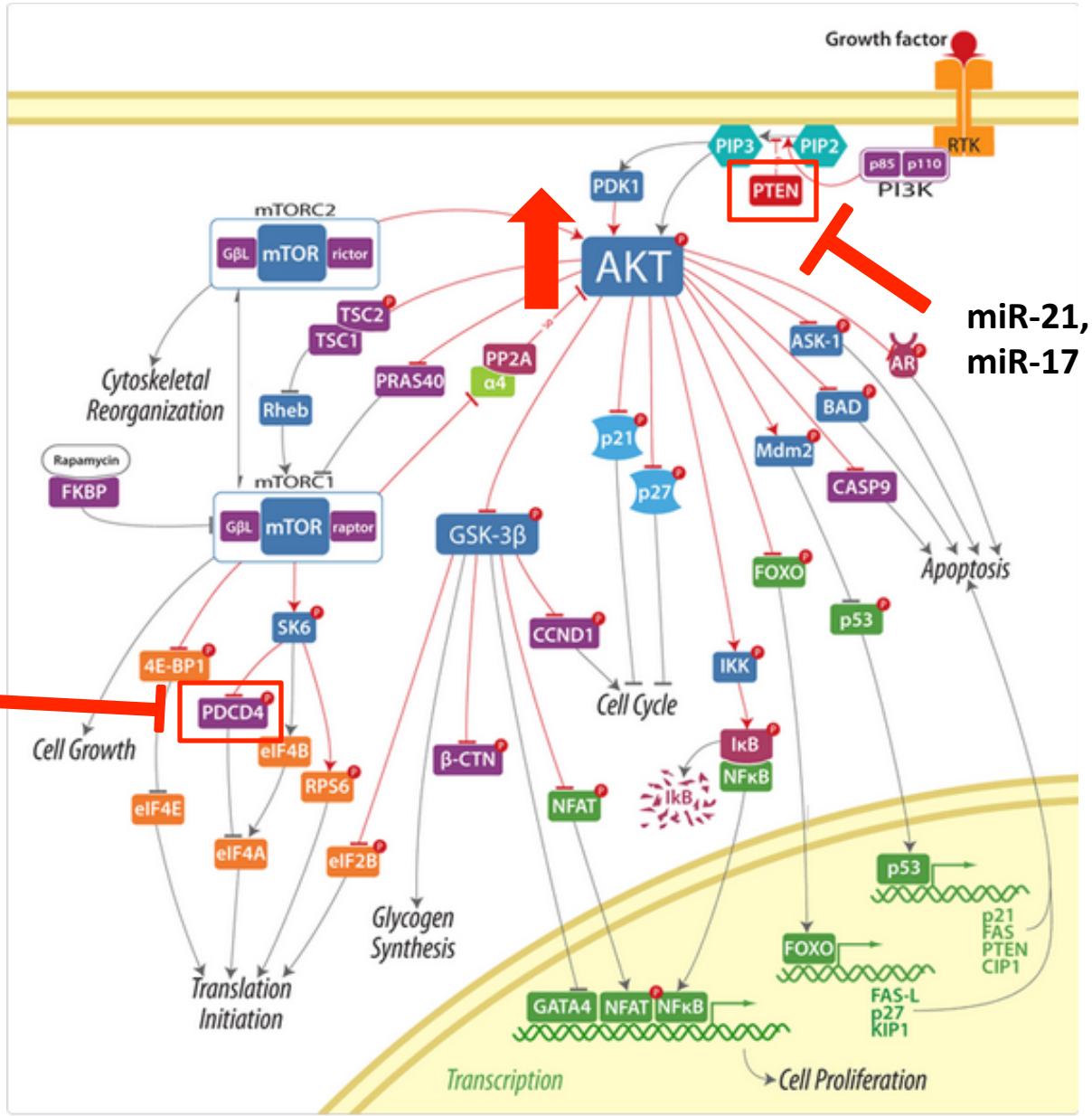
Iorio, M. V. and C. M. Croce (2012), EMBO Mol Med **4**(3): 143-159

miRNA Biogenesis and Mechanisms of Action

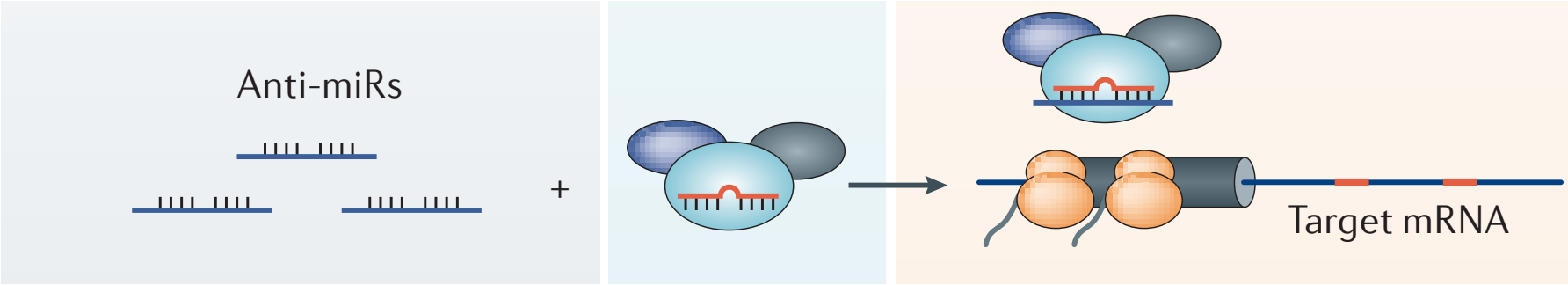


Iorio, M. V. and C. M. Croce (2012), EMBO Mol Med **4**(3): 143-159

AKT activation is an interplay between miR-21 and miR-17.

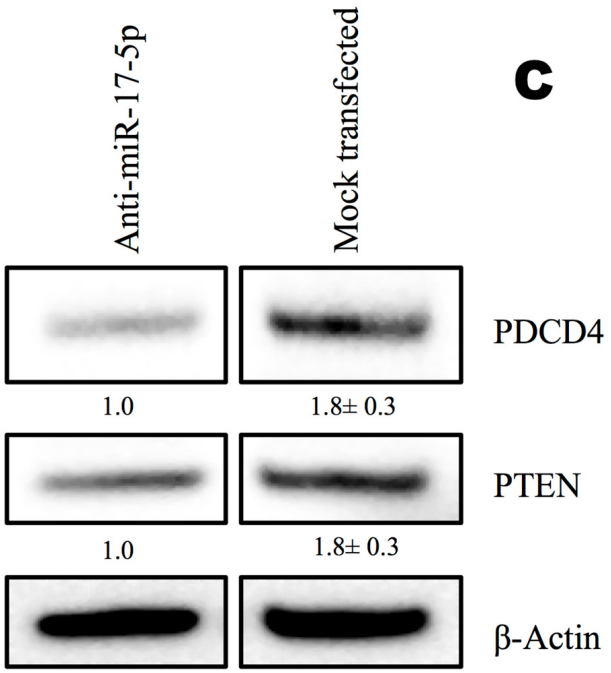


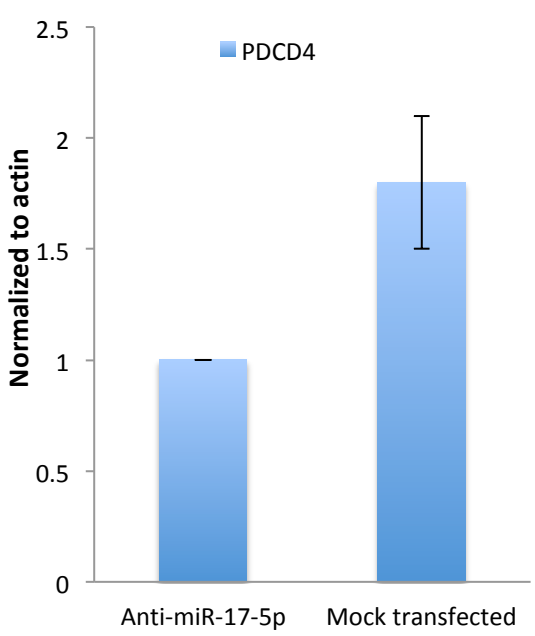
miRNA inhibition by modified oligonucleotides

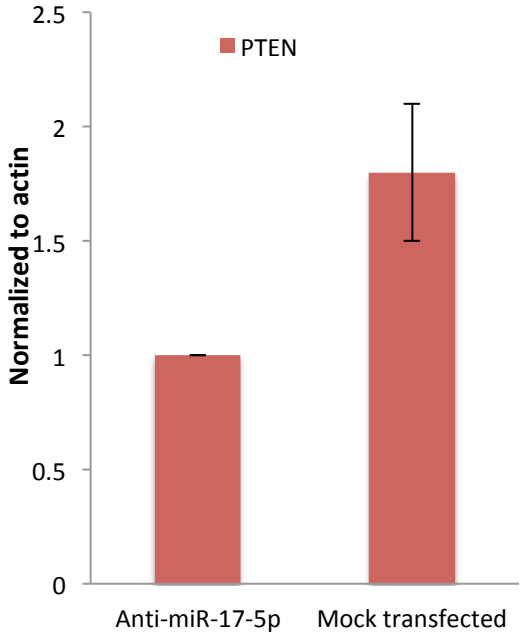


Li, Z. and T. M. Rana (2014), Nat Rev Drug Discov **13**(8): 622-638

miR-17-5p knockdown by DNA-LNA chimera unexpectedly decreased PDCD4 and PTEN protein in MDA-MB-231 cells.

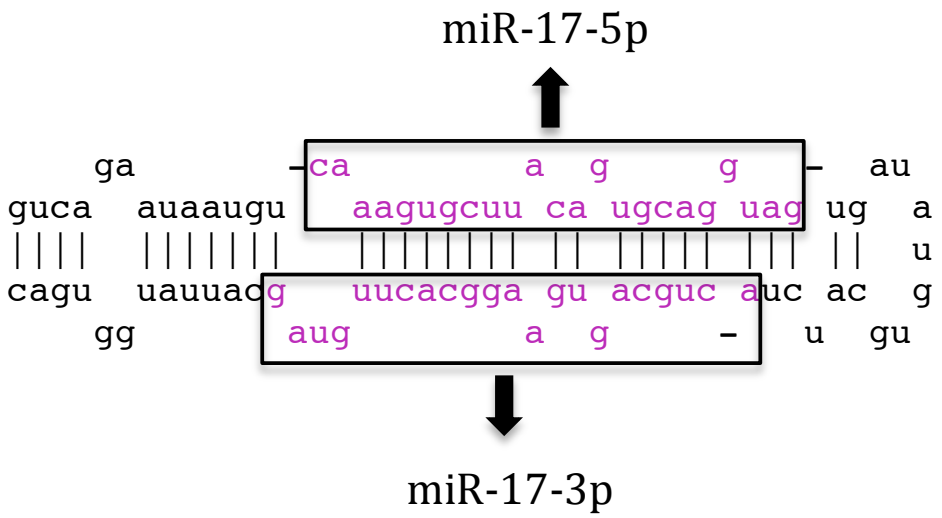






Jin, Y. Y., et al. (2015), PLoS One **10**(12): e0142574

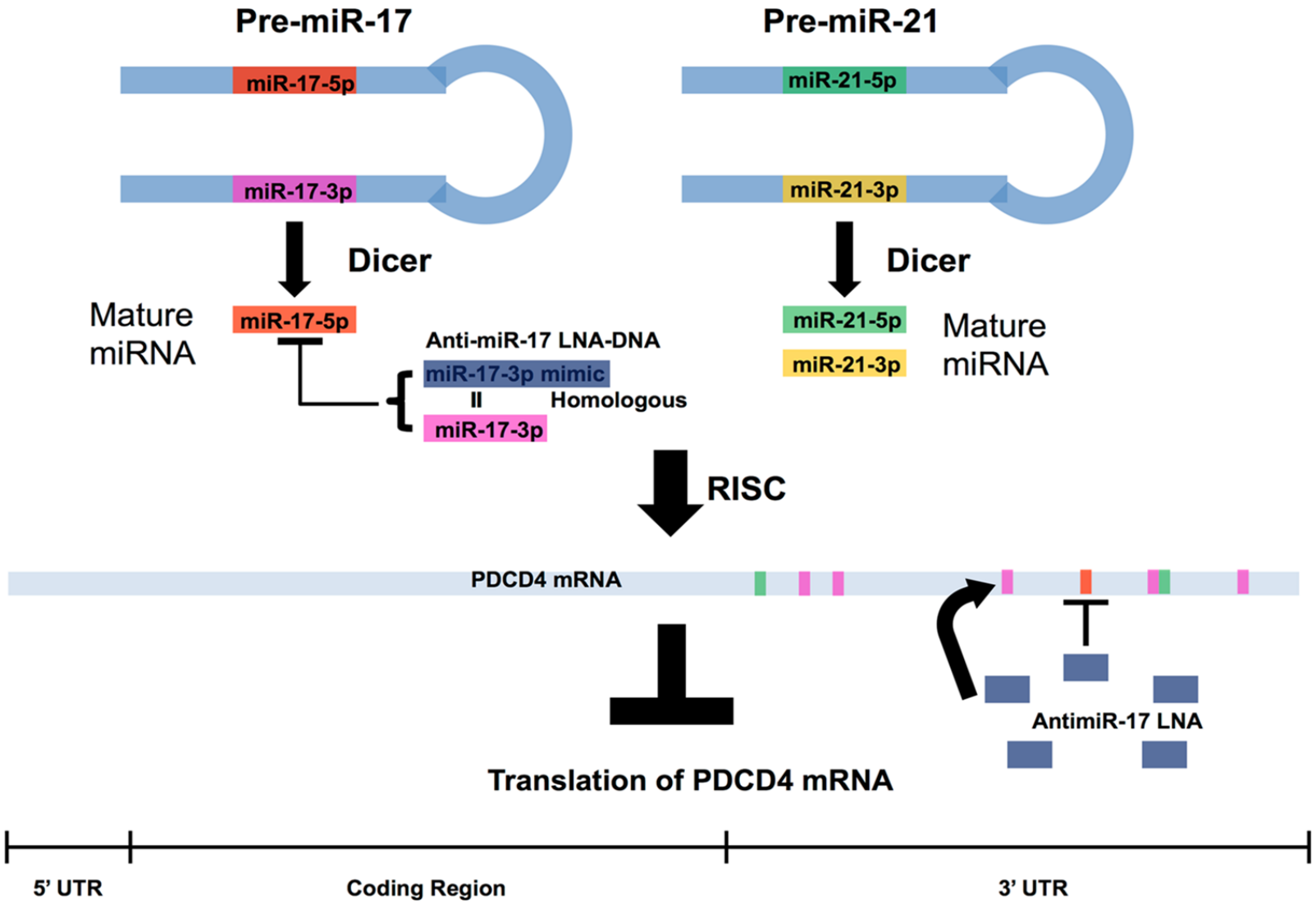
pre-miRNA structure of miR-17 revealed sequence similarity between DNA-LNA chimera and miR-17-3p passenger strand.



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

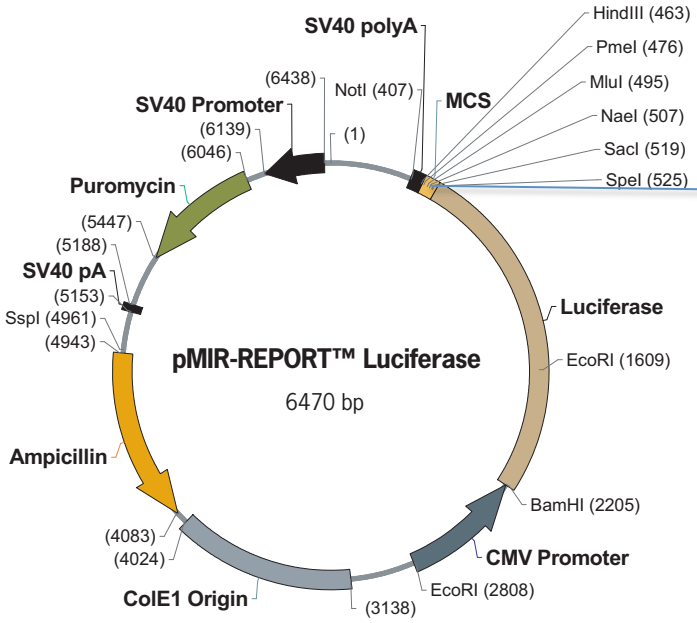
Jin, Y. Y., et al. (2015), PLoS One **10**(12): e0142574

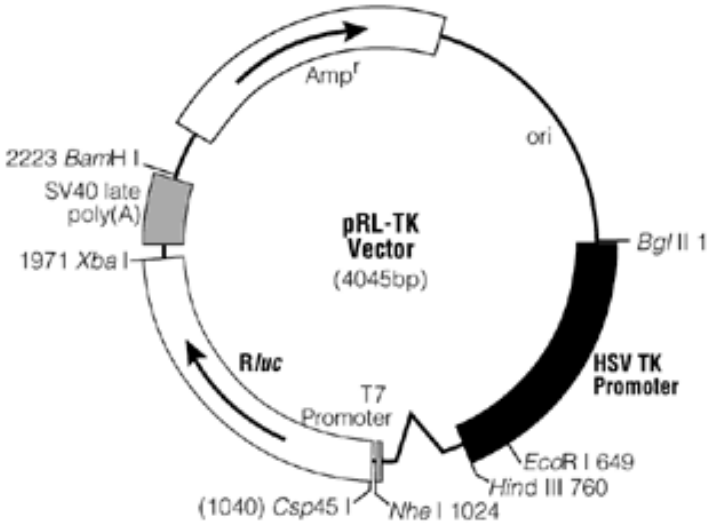
Competition between anti-miR-17-5p and miR-17-5p for inhibition of *PDCD4* mRNA



Jin, Y. Y., et al. (2015), PLoS One **10**(12): e0142574

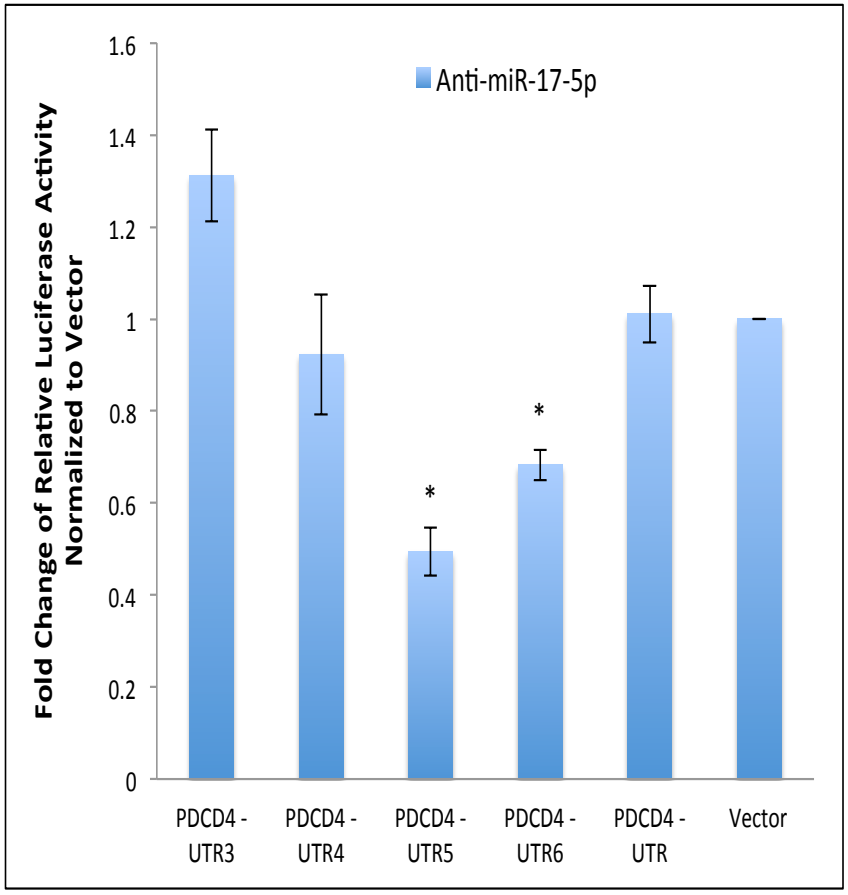
Luciferase assay system to test anti-miR-17 – mRNA interaction

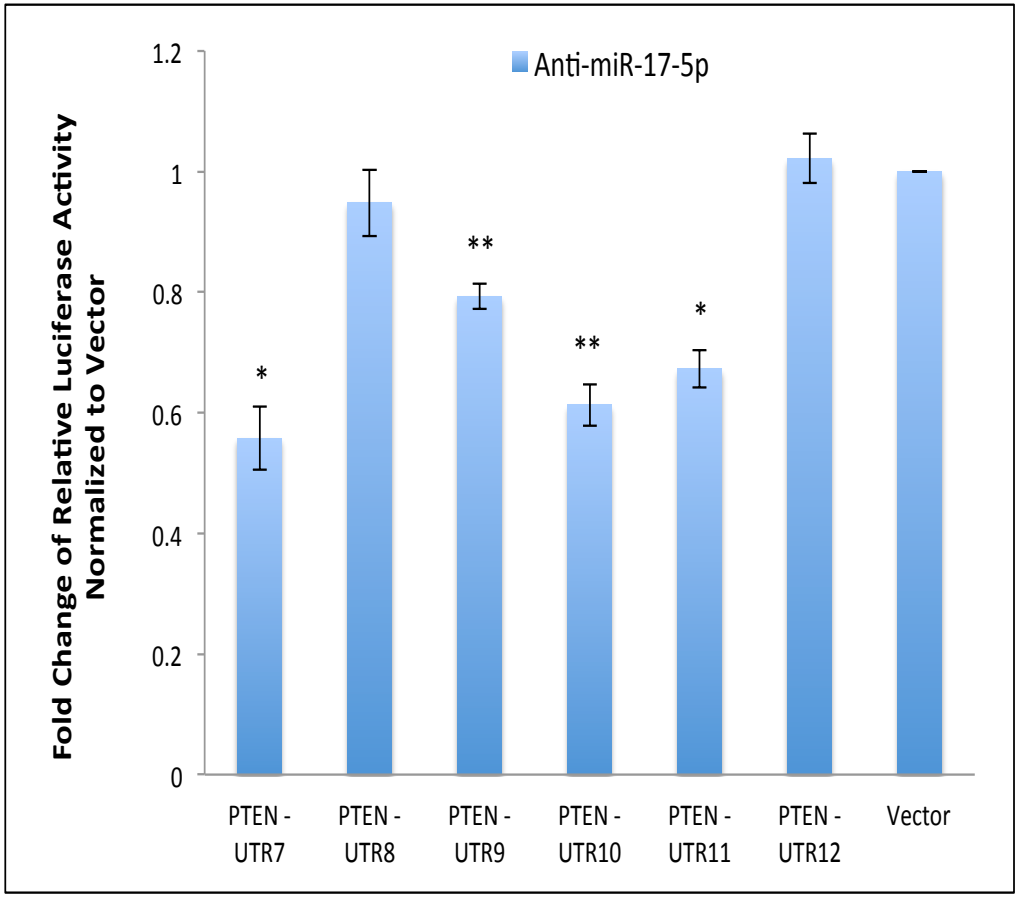




miRNA binding sites
PDCD4 3’UTR -3 – 19
PDCD4 3’UTR 325 - 346
PDCD4 3’UTR 1110 - 1136
PDCD4 3’UTR 1631 - 1652
PDCD4 3’UTR
PTEN 3’UTR 1199 - 1220
PTEN 3’UTR 5075 - 5096
PTEN 3’UTR 5828 - 5849
PTEN 3’UTR 5871 - 5892
PTEN 3’UTR 5908 - 5928
PTEN 3’UTR 6059 - 6080

Anti-miR-17-5p DNA-LNA lowered the expression of luciferase vectors containing several predicted *PDCD4* and *PTEN*'s 3'UTR target sites for miR-17-3p.

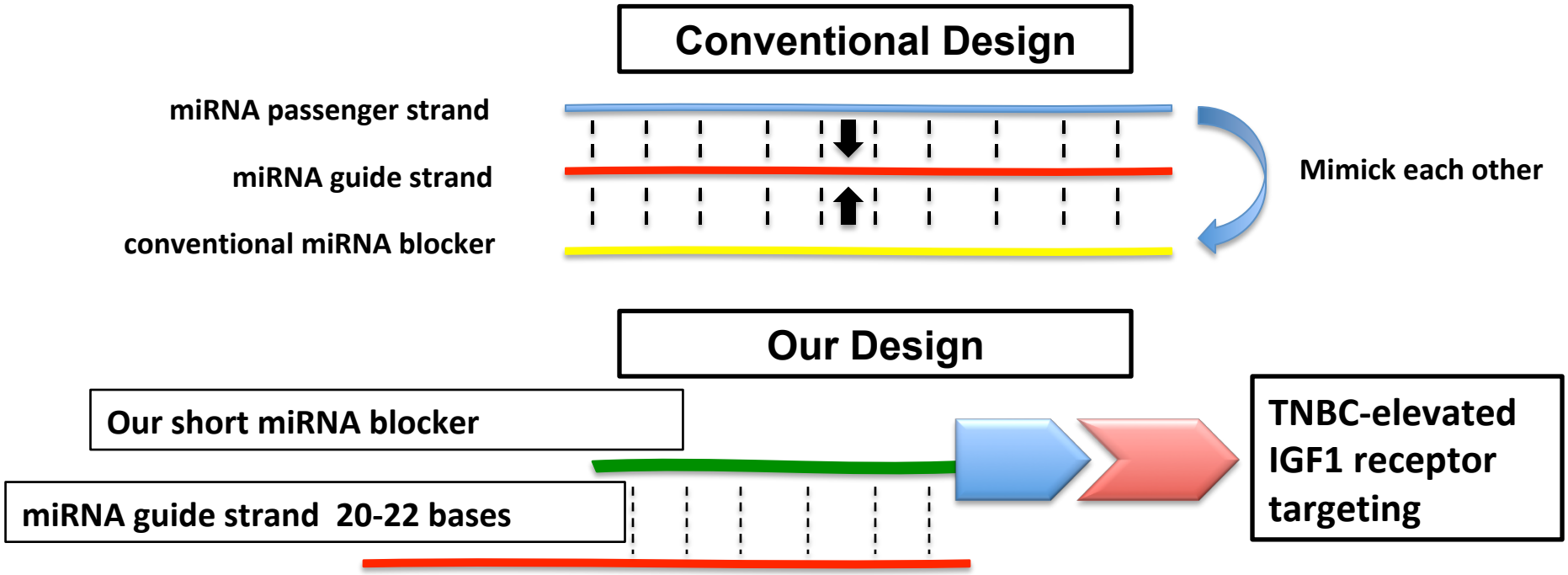




Jin, Y. Y., et al. (2015), PLoS One **10**(12): e0142574

miRNA blocker design strategy

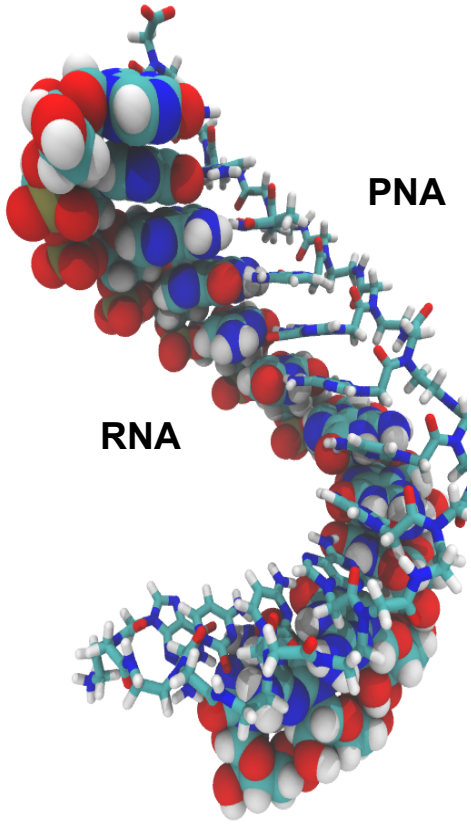
- Eliminate extra side-effects of conventional microRNA blockers
- TNBC cell-specific delivery method
- No complicated formulation, soluble in saline, intravenous route
- Next generation RNA backbones (FANA & NC-BNA vs. PNA) will elevate efficacy and potency

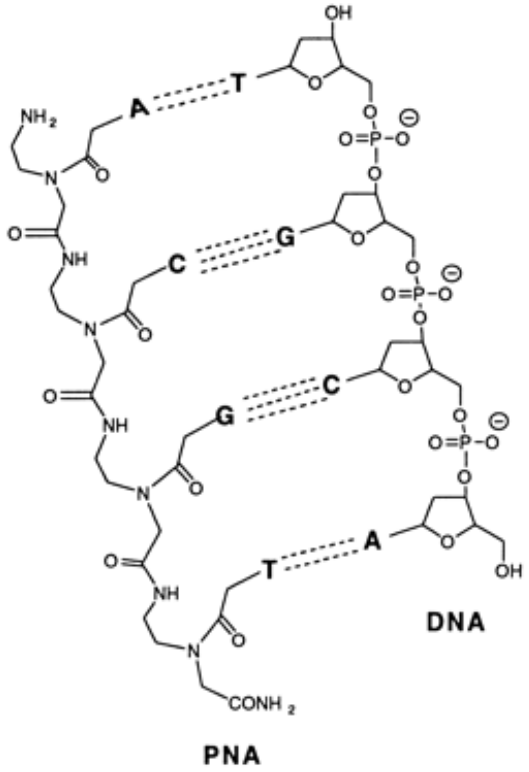


Jin *et al*. (2015) *PLoS One* **10**:10.1371/journal.pone.0142574

Nucleotide Analog - Peptide Nucleic Acids
Increasing stability, binding affinity and specificity

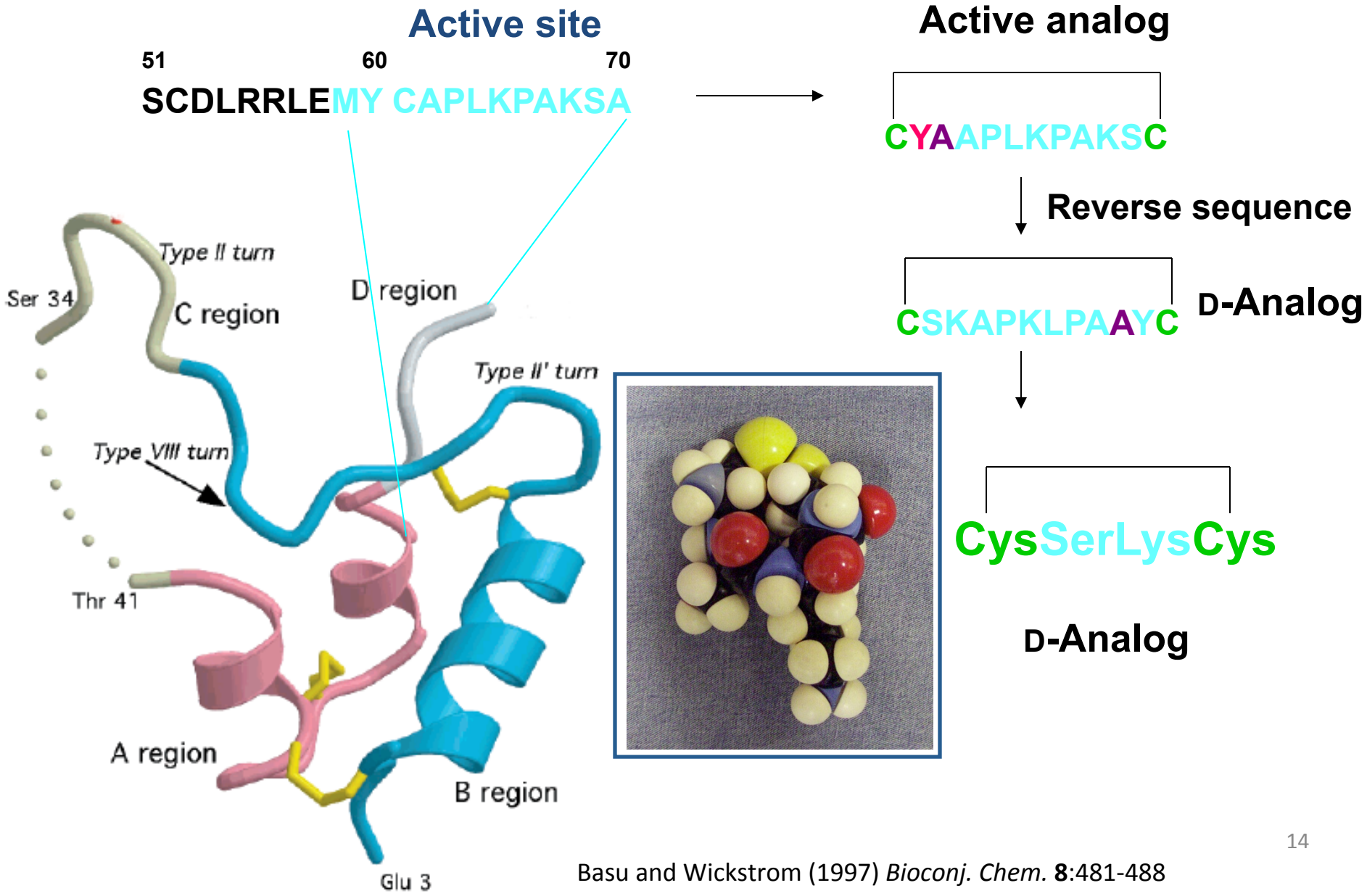
- High binding affinity to complementary DNA/RNA.
- Differentiation of single-base mismatch by high destabilizing effect.
- High chemical stability to temperature and pH.
- High biological stability to nuclease and protease.
- Good uptake via basic peptides or receptor-specific ligands
- Mice given up to 100 mg/kg dose of PNA-peptide conjugate daily did not show any irreversible toxicity (Chaubey et al., 2008).





Chaubey, B., et al. (2008), Oligonucleotides **18**(1): 9-20
Good & Nielsen (1997) *Antisense Nucl Acid Drug Dev.* **7:** 431-437

Delivery - IGF1 retro-inverso analog



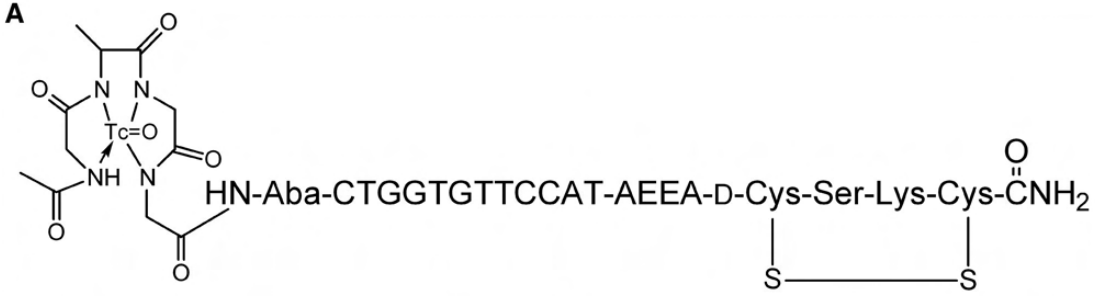
Basu and Wickstrom (1997) *Bioconj. Chem.* **8**:481-488

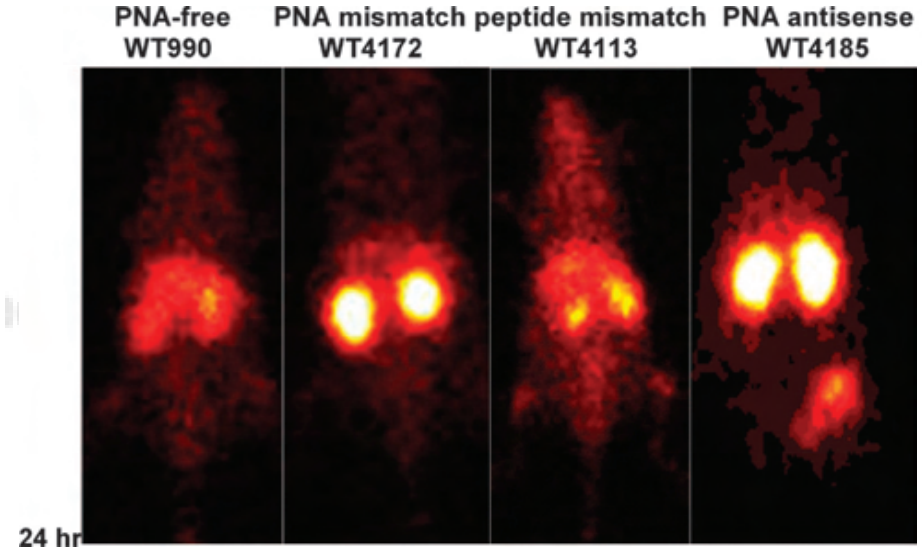
In vivo specificity of 12-mer PNA-IGF1 tetrapeptides

External Imaging of CCND1 Cancer Gene Activity in Experimental Human Breast Cancer Xenografts with ^{99m}Tc-Peptide-Peptide Nucleic Acid-Peptide Chimeras

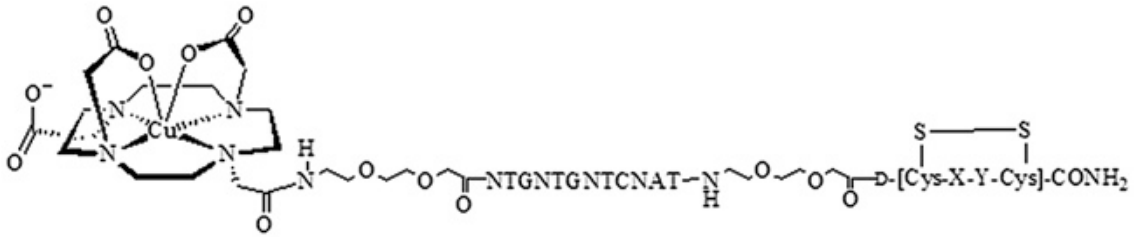
Xiaobing Tian, PhD[1]; Mohan R. Aruva, PhD[2]; Wenyi Qin, MD[3]; Weizhu Zhu, MD[3]; Kevin T. Duffy, MBA[1]; Edward R. Sauter, MD[3]; Mathew L. Thakur, PhD[2,4]; and Eric Wickstrom, PhD[1,4]

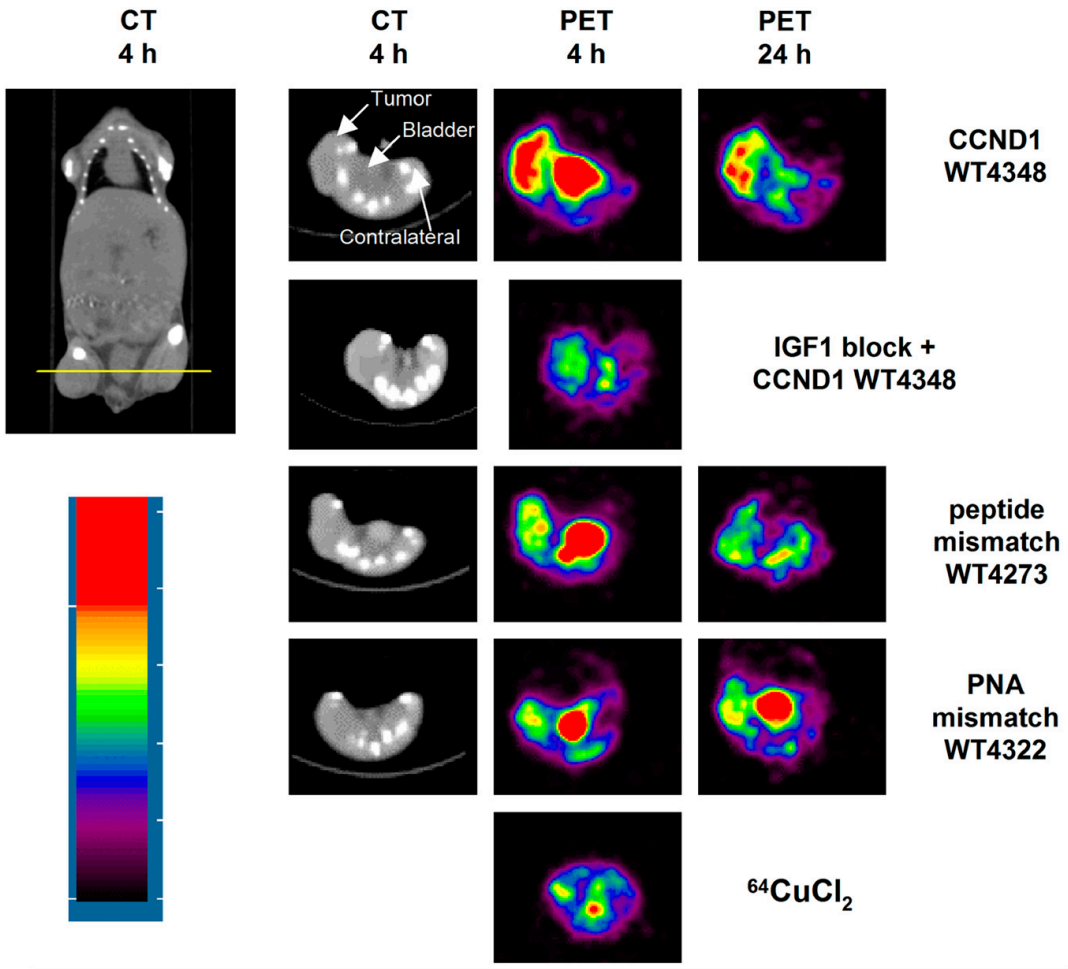
A





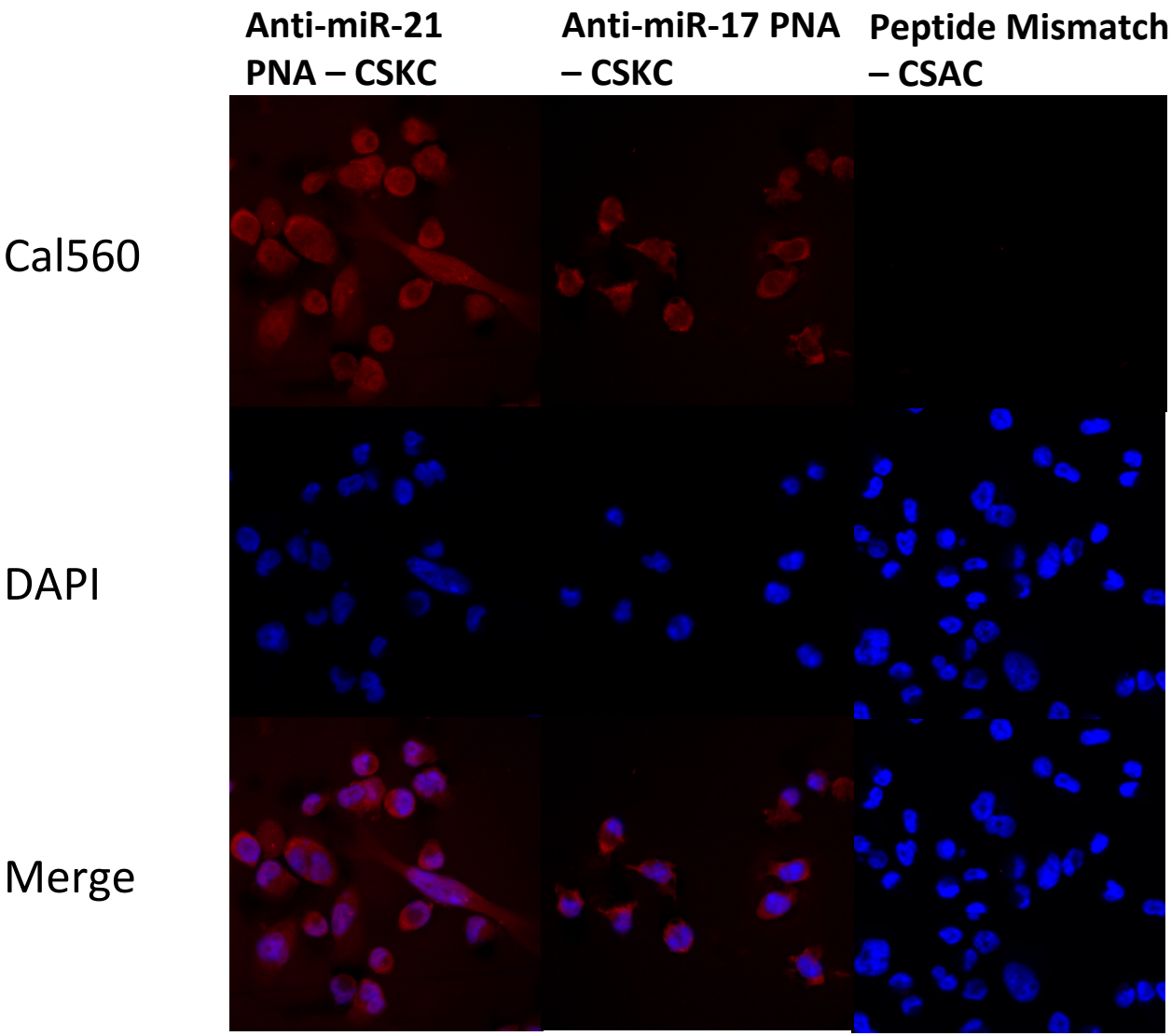
In vivo specificity of 12-mer PNA-IGF1 tetrapeptides





Tian, X., et al. (2007), J Nucl Med **48**(10): 1699-1707

MDA-MB-231 cell uptake of Cal560-Anti-miR PNA-IGF1 tetrapeptide



Cells were incubated in 200 nM of Cal560-Anti-miR PNA-IGF1 tetrapeptide and negative controls for 4 hours at 37°C in complete medium. Ex: 543 Em: 560

1 µM anti-miR PNA-IGF1 tetrapeptide elevated the expression of PDCD4 and PTEN

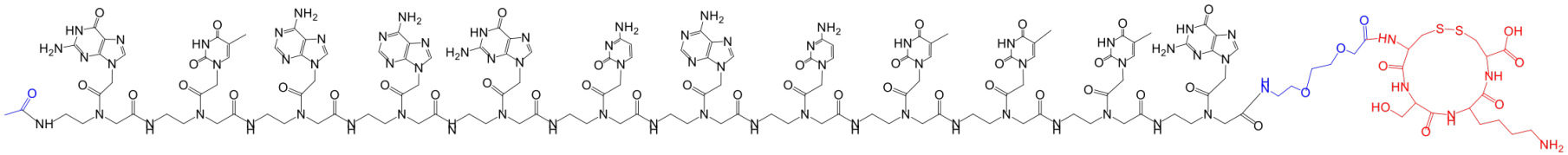
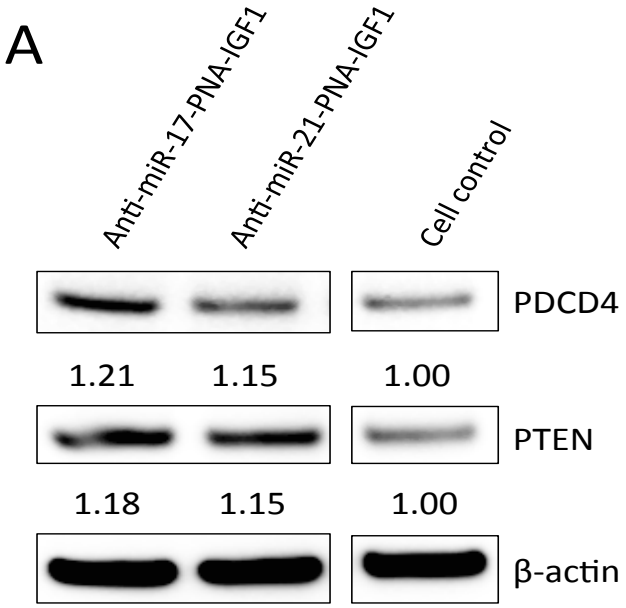
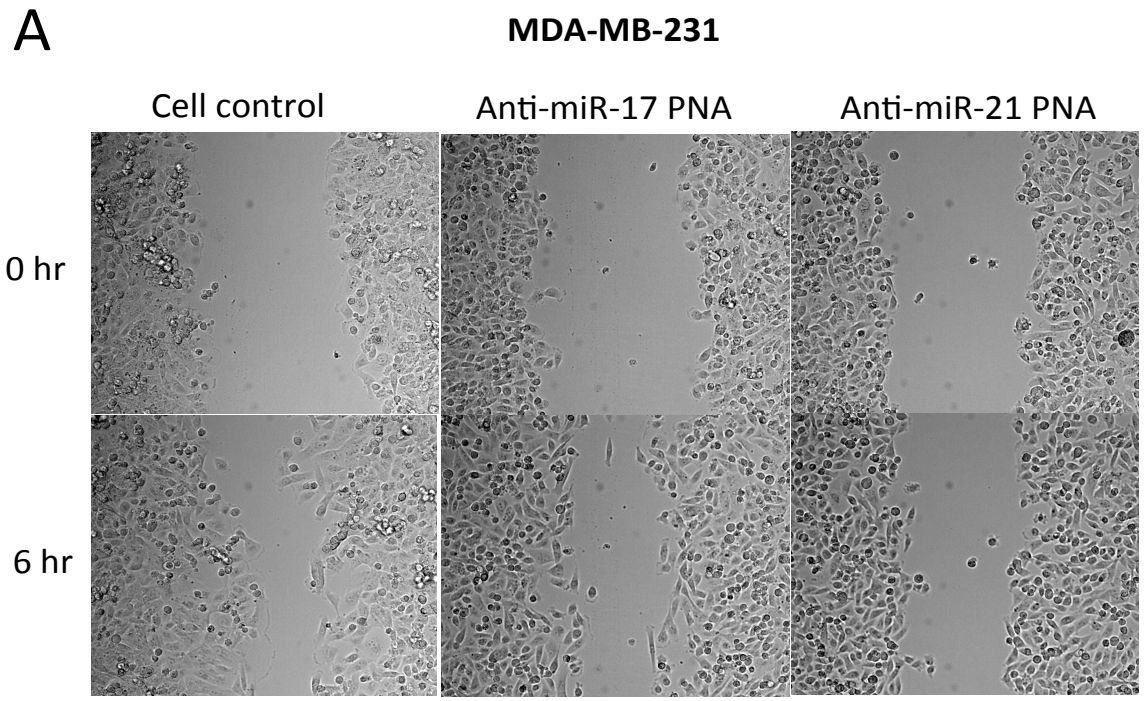
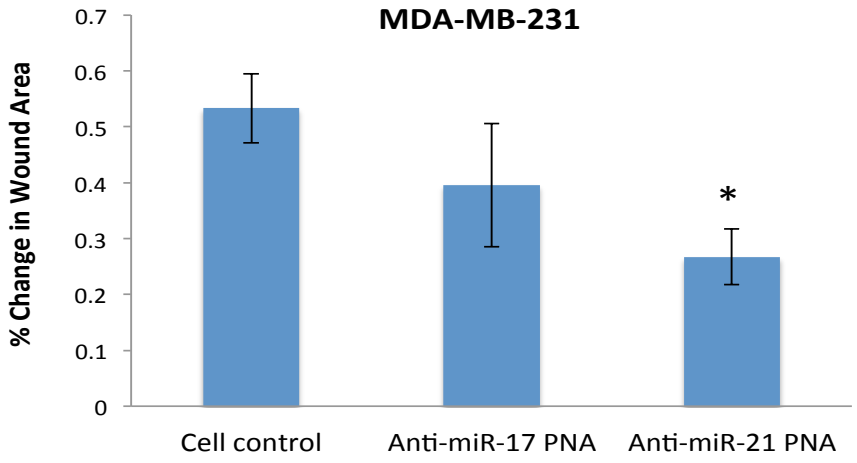


Fig. 1. PNA-AEEA-cyclo-D(Cys-Ser-Lys-Cys) blocker of miR-17-5p.



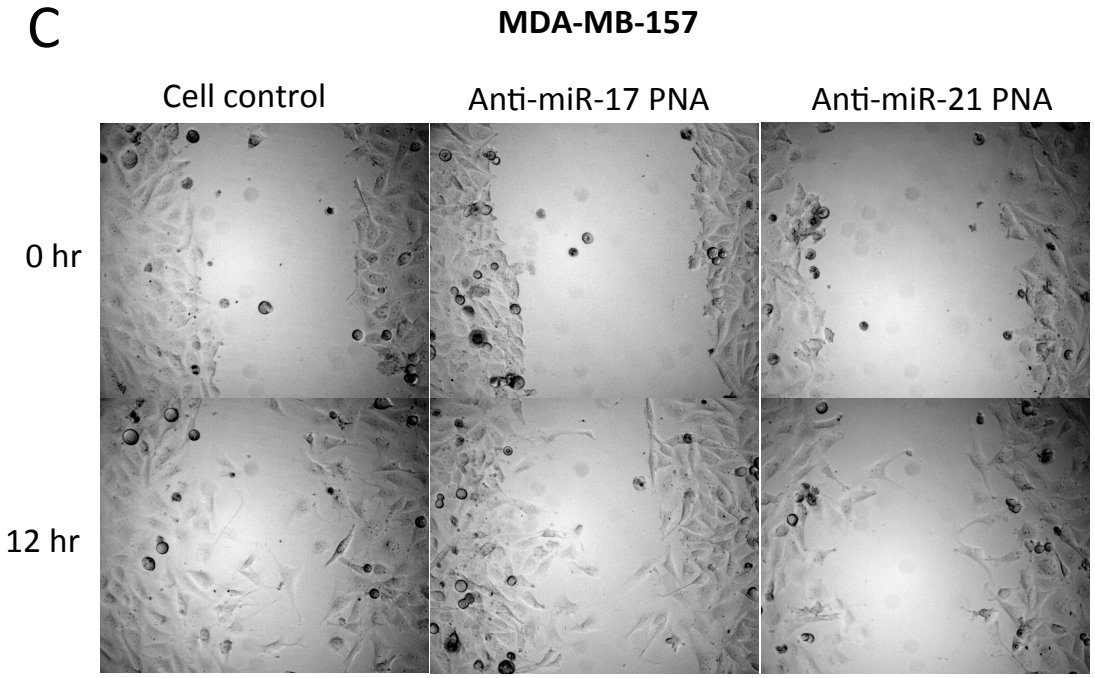
Blocking miR-21 with anti-miR-21 PNA-IGF1 tetrapeptide slowed down MDA-MB-231 cell migration.

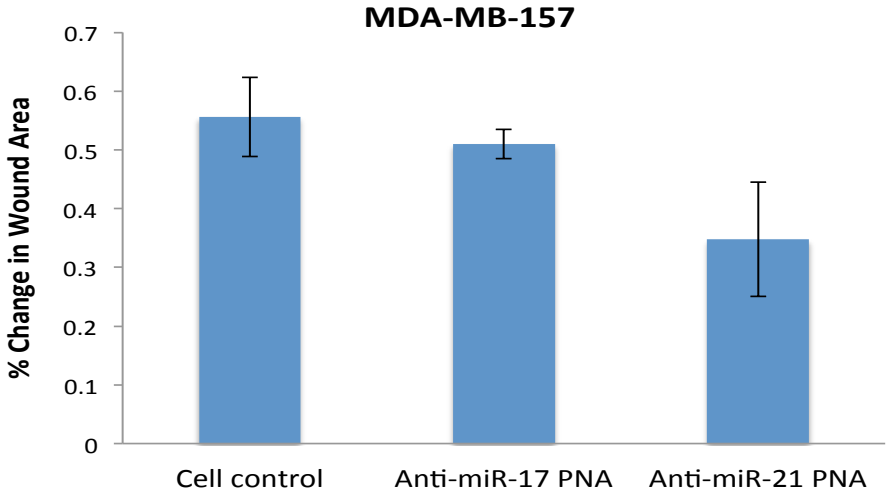




Blocking miR-21 with PNA-IGF1 tetrapeptide slowed down MDA-MB-157 cell migration.

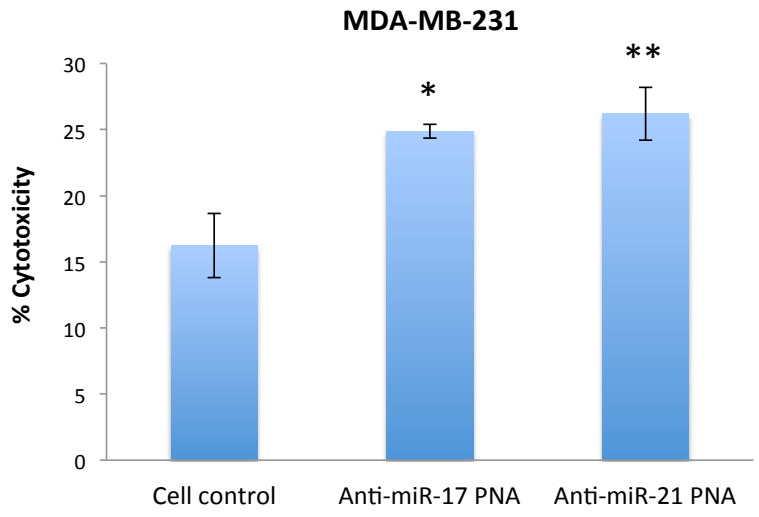
C





Blocking miR-21/17 with PNA-IGF1 tetrapeptide induced apoptosis in MSL type MDA-MB-231 and MDA-MB-157 cells.

A



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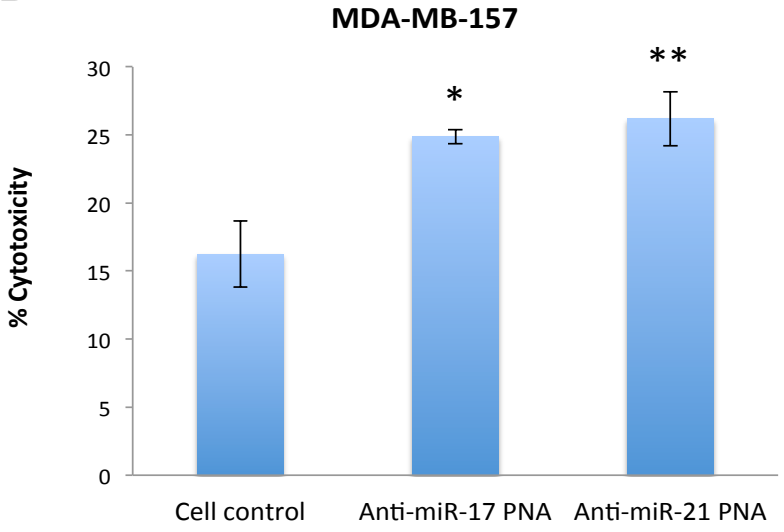
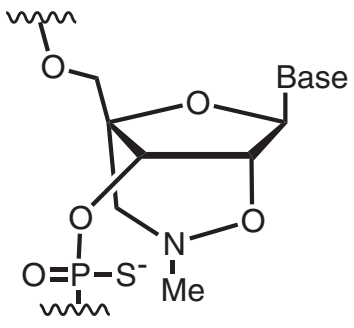
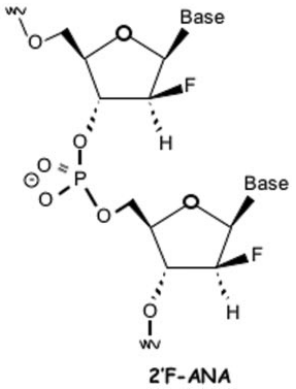


Summary

- The functional changes as a result of 1 µM PNA-IGF1 peptide treatment are modest, indicating low efficacy.
- TNBC cell lines that rely on PI3K/AKT/mTOR pathway are likely to respond to miR-21/17 blockage.
- Future antagomiRs can be optimized by:
  - Alternative oligonucleotide analog that triggers RNase H (NC-BNA, FANA)



2´,4´-BNA^{NC}



2'F-ANA

  - Increasing the length of antagomiRs without mimicking passenger strand
  - Better delivery target

Yamamoto, T., et al. (2012), Mol Ther Nucleic Acids **1**: e22
Kalota, A., et al. (2006), Nucleic Acids Res **34**(2): 451-461

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