Highly Efficient Targeting of miRNA with PNA inhibitor

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The microRNAs are approximately 22 nucleotides non-coding RNAs and are transcribed from DNA as hairpin precursors. They regulate such major processes as development, apoptosis, cell proliferation, hematopoiesis, and patterning of the nervous system. Thus, the identification of the actions of microRNAs adds new layers of complexity to our understanding of human biology. MiRNA inhibition using antisense oligonucleotide is unique and effective technique for miRNA functionalization and therapeutic targeting.

Peptide nucleic acids (PNAs) are artificial oligonucleotides with a peptide backbone. PNAs have stronger affinity and greater specificity than DNA oligonucleotides for binding to DNA and RNA. Also, PNAs are resistant to nuclease, which is essential for a miRNA inhibitor that be exposed to abundant serum and cellular nucleases. We developed PNA inhibitor and evaluated effect of PNA inhibitor on miRNA activity.

In this experiments, we confirmed that miRNA inhibition effects of PNA is over two times higher than LNA-modified DNA and 2'-O-methyl-oligonucleotide (2'-Ome). In addition, PNA showed anti-miRNA activity more rapid than LNA-modified DNA and 2'-OMe. These results demonstrated that PNAs are more powerful and effective miRNA inhibitor than LNA-modified DNA and 2'-OMe.

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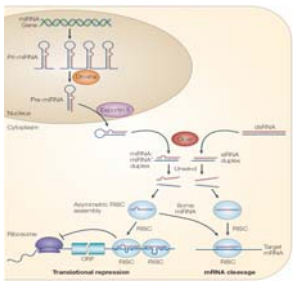
INTRODUCTION

MicroRNA

The microRNAs (miRNA) are approximately 22 nucleotides non-coding RNAs and are transcribed from DNA as hairpin precursors. They regulate such major processes as development, apoptosis, cell proliferation, hematopoiesis, and patterning of the nervous system. Thus, the identification of the actions of miRNAs adds new layers of complexity to our understanding of human biology. MiRNA inhibition using antisense oligonucleotide is unique and effective technique for miRNA functionalization and therapeutic targeting.



MicroRNA machinery
(www.openbiosystems.com/.../fig-exp-arrest.png)

Peptide nucleic acids

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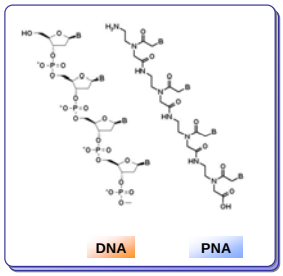

Representation of the structure of PNA & DNA

MATERIALS & METHODS

Preparation of miRNA inhibitors and plasmids

- MiRNA inhibitor sequences were obtained from the miRBase Sequence Database.
- PNAs were synthesized by Panagene.Inc, and LNA and 2'-OMe miRNA inhibitors were purchased from Exiqon and Dharmacon, respectively.
- The cells were harvested 40 hour later and luciferase activity were measured.
- For cloning of target gene into vector, we used the pGL3-control vector coding for firefly luciferase and pRL-TK vector coding for Renilla luciferase (Promega).

Cell culture and Transfection

- HeLa cell was transfected with miRNA inhibitor and target gene plasmid by Lipofectiamin 2000.
- The cells were harvested 40 hour later and luciferase activity were measured.

Cell viability

- For cell viability, MTT assay was used.

RESULTS & CONCLUSIONS

Inhibition efficiency of PNA on miRNA activity

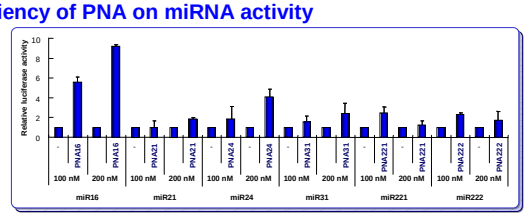

Fig. 1. Inhibition efficiency of PNA on miRNA activity. HeLa cells were co-transfected with different concentration of PNAs (100, 200 nM), pMir plasmid (miR targeting plasmid, each of pMir16, pMir21, pMir24, pMir 31, pMir221, and pMir222) and pRL-TK plasmid. After 40 hours, luciferase activity was assayed. The firefly luciferase activity was normalized to the Renilla luciferase activity.

Comparison of miRNA inhibition effects of PNA and other miRNA inhibitors

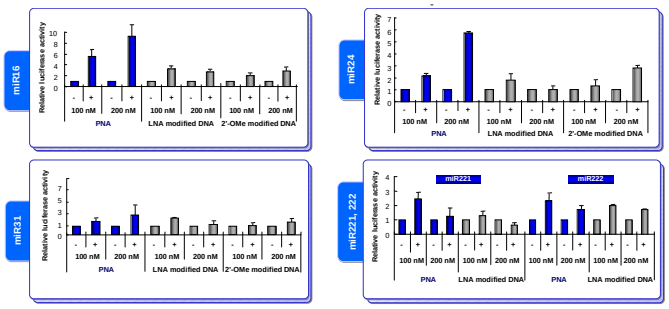

Fig. 3. Luciferase activity in HeLa cells co-transfected by lipofectamine 2000 with increasing amounts of micoRNA inhibitor were assayed. In case of miR 16 and 24, PNA inhibitor was 3 times more effective than other micorRNA inhibitors.

Effect of high concentrated PNA on cell viability

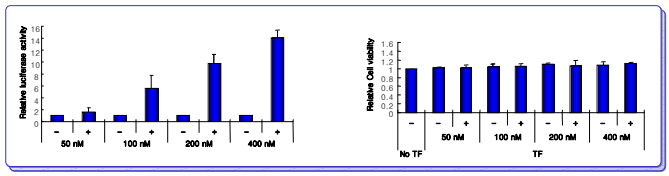

Fig. 2. MiRNA inhibition of PNA16 was increased up to 400 nM concentration of PNA. However, 400 nM PNA16 was not effect on cell viability. HeLa cell was co-transfected with PNA16 and miR16-targeted plasmid (pMir16) (+) and then were assayed with firefly luciferase activity and MTT assay. -, control; TF, transfection.

Delivery of PNA inhibitor : Evaluation of cellular uptake

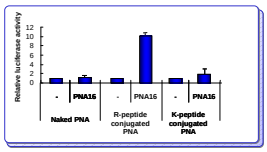

Fig. 4. R-peptide conjugated PNA is more effective in miRNA inhibition. HeLa cells were co-transfected with pMir16 and PNA16 (K-peptide conjugated PNA16 or R-peptide conjugated PNA16) by any standard transfection method.

	PNA	LNA	2'-OMe
Efficiency of miRNA inhibition	+++++	+++	+
Specificity for target miRNA	+++++	+++	++
Stability to nuclease degradation	+++++	++	+
Toxicity for cells	-	+	-
Method of penetration into cells	Transfection reagent and cell peptide conjugation (CPP)	Transfection reagent	Transfection reagent
Store	4℃ (Long-lasting)	-20℃	-20℃

The properties of PNA inhibitor are superior to other inhibitors in anti-miRNA activity

1. *MiRNA is important in gene regulation.*
2. *PNA (peptide nucleic acid) can used in experiments related miRNA regulation as antisense.*
3. *High concentration of PNA is not effect on cell viability.*
4. *PNA has higher inhibition of miRNA than other miRNA inhibitor.*
5. *PNA is more powerful material in miRNA inhibition.*

For more detailed information,
please contact us at www. panagene.com

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