Novel Cell - Penetrating Drug Delivery System for siRNA

JenKem Technology

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ABSTRACT

Experience has shown that siRNA drugs, while potentially very useful, are easily degraded by nucleases in vivo, and their relatively high molecular weight, negative charge and hydrophilicity inhibit their ability to permeate through cell membranes [1, 2].

To circumvent these deficiencies, a novel drug delivery system consisting of a cell penetrating peptide (LMWP) linked to activated PEGs (polyethylene glycol polymers) has been developed for siRNA delivery.

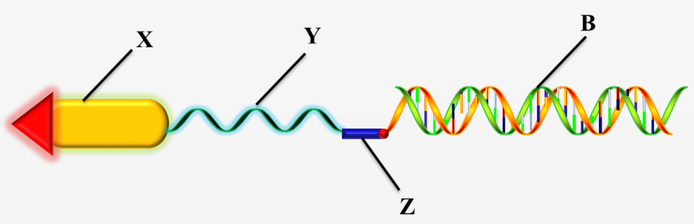

Figure 1. Illustration of LMWP-PEG-RNA Conjugate

X = LMWP
Y = PEG
B = RNA
Z = Cytosol-Cleavable Linkage

The addition of the PEG improves the drug's bioavailability by preventing the self-assembly and formation of intra-molecular hairpin structures between the cell penetrating peptide and siRNA [3].

In vitro studies confirm successful cellular uptake of siRNA and high gene-silencing efficacy using this novel cell penetrating peptide-PEG drug delivery system.

METHODS

LMWP-PEG was first conjugated with FITC labeled anti-EGFP siRNA. Cell uptake studies for LMWP-PEG-RNA conjugate with anti-EGFP siRNA were carried out on MDA-MB-231 cells using a confocal microscope.
The gene silencing down-regulating effect by anti-EGFP siRNA was assessed by laser scanning microscopy using EGFP over-expressed MDA-MB-231-EGFP cells.

INTRACELLULAR UPTAKE AND LOCALIZATION OF siRNA

As seen in Figure 2, anti-EGFP siRNA alone cannot penetrate the cells membrane. The fluorescence intensity of LMWP-PEG-RNA inside the cells is very strong, suggesting that the cellular uptake after conjugation with this novel drug delivery system is significant.

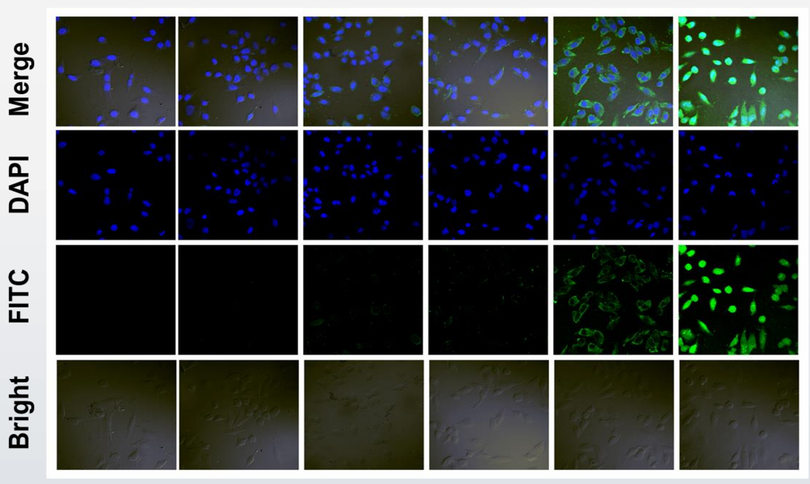

Figure 2. Intracellular uptake study.
(1) PBS (2) anti-EGFP siRNA (3) Lipofecter and anti-GFP siRNA complex (4) LMWP-PEG and anti-EGFP siRNA mixture (molar ratio: 1:1) (5) LMWP and anti-EGFP siRNA mixture (molar ratio: 1:1), and (6) LMWP-PEG-anti-EGFP siRNA conjugate.

GENE SILENCING EFFICACY OF siRNA

As seen in Figure 3, LMWP-PEG-RNA has a high membrane penetrating ability and significantly inhibits target gene expression.

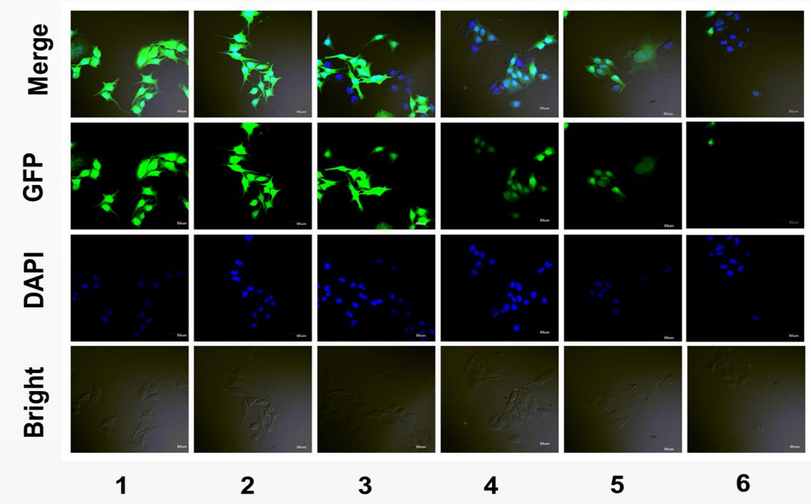

Figure 3. Gene silencing study
(1) PBS, (2) siRNA negative control, (3) anti-EGFP siRNA, (4) Lipofecter and anti-EGFP siRNA complex, (5) LMWP and anti-EGFP siRNA mixture (molar ratio: 1:1), and (6) the LMWP-PEG-anti-EGFP siRNA conjugate.

CONCLUSIONS

In vitro findings confirmed that our novel LMWP-PEG drug delivery system yields successful cellular uptake of siRNA and high gene-silencing efficacy.

LMWP-PEGs can be employed to deliver oligonucleotides for gene therapy, and other drug molecules that cannot penetrate the cell membrane on their own.

REFERENCES

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