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Controlled Intracellular Trafficking and Gene Silencing by Oligonucleotide-Signal Peptide Conjugates

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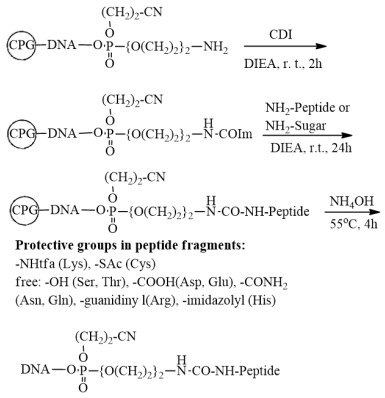
Abstract

In the present study, we investigated the intracellular delivery using some hybrid peptides as transfection reagents and the silencing effect of siRNA targeting human telomerase reverse transcriptase (hTERT) mRNA in 3 human cancer cell lines, Jurkat and HeLa [1,2,3]. The complex of siRNA and a specific amphiphilic peptide (Pfect7) or its hybrid with an intracellular transport signal peptide could be effectively taken up into cells. The complex also showed a high silencing effect against hTERT mRNA

. Combination of siRNA-nuclear export signal (NES) peptide conjugates and the amphiphilic peptides suppressed the expression of BCR/ABL chimeric gene in chronic myelogenous leukemia (CML) cell line K562 up to 95.2 % [4]. The amphiphilic peptides and their hybrids showed almost no cyto-toxicity and protected siRNA against intracellular nuclease digestion in 10% FBS (half life time was over 48h).

Synthesis of Oligonucleotide-Peptide Conjugates by SPFC



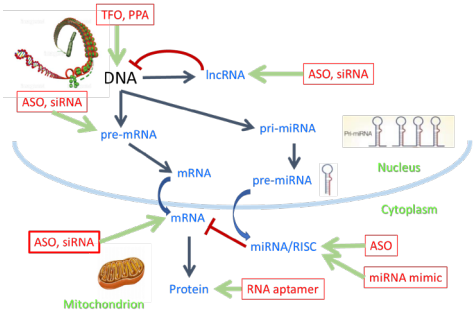
R-CONHCH$_2$CH$_2$OCH$_2$CH$_2$OPO$_3$-5'-CAGTTAGGGTTAG-3'

C1: R = Ac-GPKKKRKVGK- NH- (SV40 T antigen NLS)
C1f: R = FITC-GPKKKRKVGK- NH- (SV40 T antigen NLS)
C2: R = Ac-GRKKRRQRRRPPGGK- NH- (HIV-1 Tat NLS)
C2f: R = FITC-GRKKRRQRRRPPGGK- NH- (HIV-1 Tat NLS)
C3: R = - NH- ANSAAFEDLRVLS-OH (Influenza Virus nucleoprotein NLS)
C4: R = Ac-LPPLERLTLGK- NH- (HIV-1 Rev NES)
C4f: R = FITC-LPPLERLTLGK- NH- (HIV-1 Rev NES)
C5: R = - NH - ALRALLRALLRAL-OH (designed)
C5f: R = - NH - ALRALLRALLRAL-OH (designed)
C6: R = - NH - ARLRLRLRLRL-OH (designed)
C7: R = galactosamine

R-CONHCH$_2$CH$_2$OCH$_2$CH$_2$OPO$_3$-5'-s(CAGTTAGGGTTAG)-3'

C8: R = Ac-GPKKKRKVGK- NH- (SV40 T antigen NLS)
C8f: R = FITC-GPKKKRKVGK- NH- (SV40 T antigen NLS)

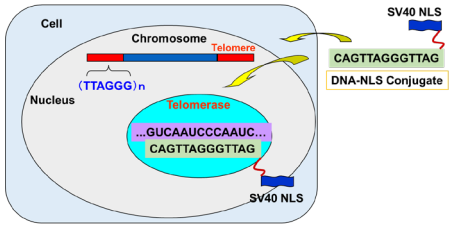
[1] Masayuki Fujii *et al., Org. Lett.*, **2003**, 5, 2623-2626.
Bioorg. & Med. Chem. Lett., **2005**, 15, 167-170.
Org. & Biomol.Chem., **2005**, 3, 3257 ? 3259.
Fronteers in Org. Chem., **2005**, 1, 229-241.
Bioorg. & Med. Chem. Lett., **2007**, 17, 6576-6578.
Curr. Org. Chem., **2009**, 13, 1366-1377.



Nucleic Acid Therapeutics and Their Targets

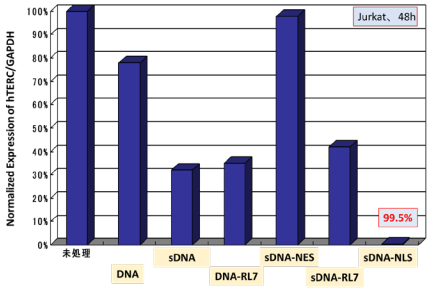
Nucleic Acid Therapeutics should
(1) be stable enough chemically and biologically, inside and outside the cells,
(2) be delivered to the targeted tissue,
(3) be taken up into cells effectively,
(4) be localized in the specific intracellular organs,
(5) bind to the target molecule with enough affinity and specificity,
(6) not be toxic,
(7) not cause undesirable effect (off-target effect),
(8) not induce immunoresponse,
. . . .

Inhibition of hTERC by Antisense DNA-NLS Conjugate



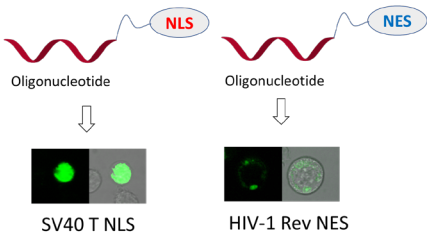
Ac-GPKKKRKVGK-εNH-CONHCH$_2$CH$_2$OCH$_2$CH$_2$OPO$_3$-5'-s(CAGTTAGGGTTAG)-3'
(SV40 T antigen NLS)

Inhibition of hTERC by Antisense DNA-NLS Conjugate

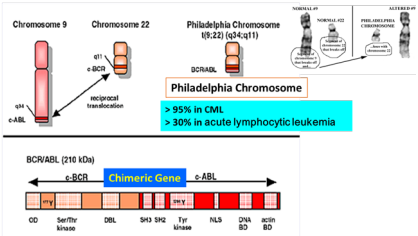


[2] M. Fujii, *et al.*, Org. & Biomol.Chem., 2005, 3, 3257-3259.
[3] Fujii M et al. (2020) Nucleosides, Nucleotides and Nucleic Acids 39(1-3):407-425.

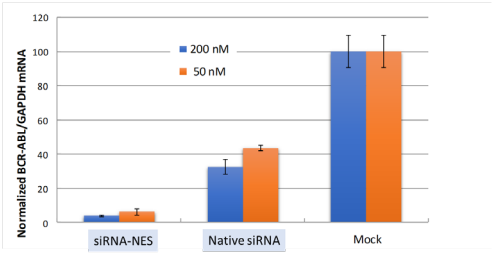
Controlled Delivery of DNA-Signal Peptide Conjugates



Chronic Myelogenous Leukemia (CML)



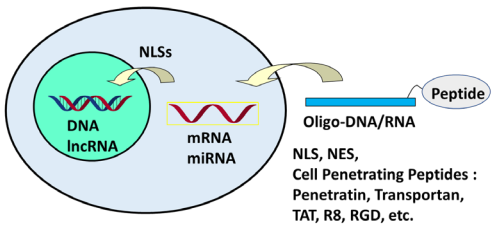
Target sequence of bcr/abl mRNA(355-390)
5'-ggauuuaagcagaguucaa/aagcccuucagcggcca-3'



Silencing of bcr-abl mRNA by siRNA6 (HIV1 REV NES) in K562.

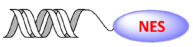
siRNA suppressed bcr-abl mRNA expression in K562 cells. Normalized bcr-abl/GAPDH mRNA levels were measured in 24 h after transfection with Lipofectamine 2000™.

Delivery and Gene Silencing by Oligonucleotide-Peptide Conjugates



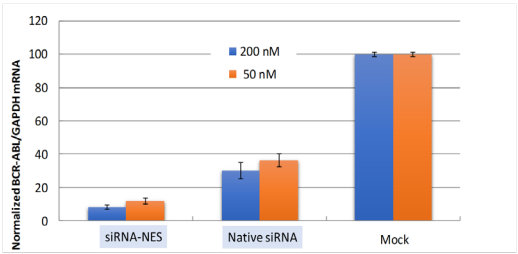
NLS, NES,
Cell Penetrating Peptides :
Penetratin, Transportan, TAT, R8, RGD, etc.

Specific Suppression of BCR-ABL mRNA by siRNA-NES Conjugates Targeting b3a2 Transcripts



Target sequence of bcr/abl mRNA(355-390)
5'-ggauuuaagcagaguucaa/aagcccuucagcggcca-3'

siRNA
Sense; 5'-NES-GCAGAGUUCAAAAGCCCUUTT-3'
Antisense; 5'-AAGGGCTTTTGAACUCUGCTT-3'
NES Peptides:
C1; -NHCH$_2$CH$_2$CH$_2$CO-LPVLENLTL-OH (TFIIIA NES)
C2; AcNH-LPPLERLTL-K(εNH-)G-OH (HIV-1 REV NES)



Silencing of bcr-abl mRNA by siRNA5 (TFIIIA NES) in K562.

siRNA suppressed bcr-abl mRNA expression in K562 cells. Normalized bcr-abl/GAPDH mRNA levels were measured in 24 h after transfection with Lipofectamine 2000™.

[4] M. Fujii, *et al., Nucleic Acid Therapeutics*, 2017, 27(3), 168-175.

COI: We have no conflict of interest to disclose for this presentation.