

Knockdown of BACE-1, an Alzheimer's Disease Drug Target, with NIMT[®]FeOfection|PURPLE

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Introduction

Small interfering RNA (siRNA) has become a widely used tool over the past years in the study of gene function and shows potential for drug development. However, *in vivo* siRNA delivery is the major obstacle that has to be overcome. The commercial cationic lipid based transfection reagents are toxic to the cells and are not compatible for *in vivo* application.

Alzheimer's disease (AD) is the most common cause of dementia. The amyloid- β (A β) peptide is believed to play a key role in the pathogenesis of AD (1, 2). The enzyme β -secretase 1 (BACE-1) is responsible for the cleavage of transmembrane protein APP and production of A β 1-42 peptides. A β 1-42 is the principal component of amyloid plaques in AD patients. The utilization of siRNA is one approach of interfering with BACE-1 activity and thereby reducing formation of A β peptides. Here, a novel transfection agent, NIMT[®]FeOfection|PURPLE, was evaluated for delivery of siRNA into HEK-293T cells.

Materials and Methods

Cell Culture

HEK-293T (Human Embryonic Kidney) cells were cultured in MEM (minimal essential medium) supplemented with 10% (FBS) and 1% streptomycin/penicillin in 37°C at 5% CO₂. Twenty-four hours prior to transfection, cells were seeded in 6-well plates in medium without antibiotics.

Transfection of siRNA

siRNAs used: BACE1 siRNA (Invitrogen), Non-targeting control siRNA (Ambion). siRNAs and NIMT[®]FeOfection|PURPLE were diluted in 0.2 ml OptiMEM (Invitrogen). The samples were mixed and the resulting complexes were incubated 20 min at RT before addition to cells. 4-6 hours post transfection media was replaced with new media containing 1% streptomycin/penicillin. Transfection with Lipofectamine 2000

(Invitrogen) was performed according to the manufacturer's protocol.

Quantitative Real Time PCR

Knockdown of BACE-1 mRNA was quantified with Real Time PCR 24 hrs post transfection. RNA was extracted using RNeasy Miniprep Kit (Qiagen).

Results and Discussion

High Transfection Efficiency

The fluorescent labeled siRNA was readily taken up by cells when using NIMT[®]FeOfection|PURPLE as transfection agent as shown in Figure 1.

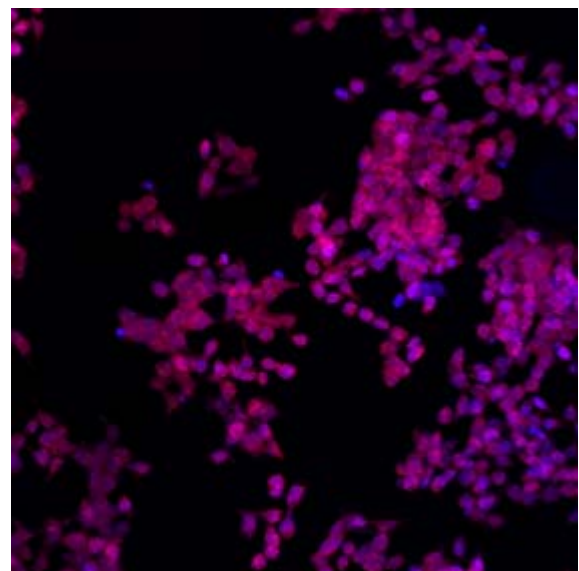


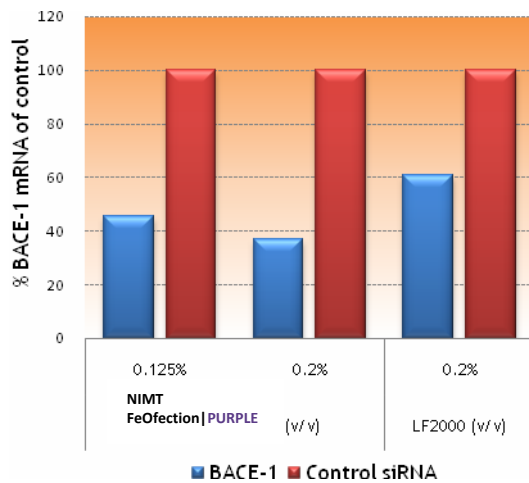
Figure 1: Confocal microscope image of HEK-293T cells transfected with fluorescent siRNA using NIMT[®]FeOfection|PURPLE. The nuclei are stained with Hoechst 33342.

Effective Gene Silencing with NIMT[®]FeOfection|PURPLE

The BACE-1 mRNA could be successfully silenced using NIMT[®]FeOfection|PURPLE for

BACE-1 siRNA delivery. Lowering the transfection agent concentration still yielded a considerable knockdown of the target gene (figure 2). A 55% knockdown was observed with 0.125% NIMT[®]FeOfection|PURPLE which was only slightly lower than when using 0.2% NIMT[®]FeOfection|PURPLE (63% knockdown).

Figure 2: The knockdown of BACE-1 mRNA using NIMT[®]FeOfection|PURPLE or Lipofectamine 2000 as transfection agent was quantified with real time PCR.



References

1. Sinha S, et al., *Recent advances in the understanding of the processing of APP to beta amyloid peptide*. Ann N Y Acad Sci (2000) **920**:206-8
2. Selkoe DJ and Scenk D, *Alzheimer's disease: Molecular understanding predicts amyloid-based therapeutics*. Annu Rev Pharmacol Toxicol (2003) **43**:545-584