AS-ODN delivery into mammalian cells using high hydrostatic pressurized cationic liposome

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Statement of Purpose: Regulation of gene expression using oligonucleotides, such as antisense oligodeoxynucleotide (AS-ODN), small interfering RNA (siRNA) and micro RNA (miRNA), is one of attractive methods for gene therapy [1]. It is important to deliver oligonucleotides into cells effectively, and to inhibit target-specific gene expression. Cationic compounds, such as cationic polymers and cationic lipids (CLs), were used to introduce oligonucleotides into cells because the stable and small complexes were formed with oligonucleotides. Among them, CLs are mainly used for effective delivery. However, the efficiency and selectivity of gene suppression are insufficient to clinical usage. Previously, we reported that the transfection efficiency was increased by using the pressurized plasmid DNA/CL complexes [2]. In this study, we investigated the effect of HHP treatment for AS-ODN/CL complex on AS-ODN delivery.

Methods: AS-ODNs were designed to inhibit expression of firefly luciferase (luc2) (Figure 1). Cationic liposome consisting of DOPE and DOTMA was prepared according to the extrusion method [3]. The complexes of AS-ODN and CL at various ratios of C/A ratios were prepared, and then pressurized hydrostatically at various atmospheres (~10,000 atm) for 10 min (HHP treatment). After removal of pressure, the pressurized AS-ODN/CL complexes were subjected to DLS measurement and AFM and TEM observation. The stability of AS-ODN/CL complexes with and without the HHP treatment was evaluated by fluorescence measurement using fluorescent hydrophobic probes (Laurdan and pyrene). The efficiency of gene suppression of the HHP treated AS-ODN/CL complexes was assessed for HEK293 cells expressing luc2 stably.

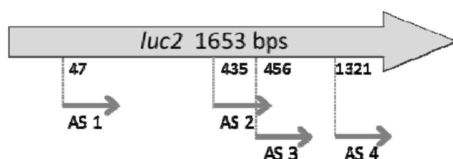

Figure 1. Target site of AS-ODNs used

Results: The HHP-treated AS-ODN/CL complexes were added to HEK293 cells expressing luc2 stably. The luciferase activity was measured by using luminometer. Table 1 shows the efficiency of gene silencing for transfection of AS-ODNs using the AS-ODN/CL complexes with/without HHP treatment (10,000 atm). Although the levels of gene suppression were different between AS-ODNs used, the efficiency of gene silencing was increased by using the HHP-treated AS-ODN/CL complexes compared to the non-pressurized AS-ODN/CL complexes.

Table1. Gene silencing by ONs delivered into HEK293 expressing luc2 using HHP-lipoplex.

ONs	Relative to control	
	Non-treated lipoplex	HHP-treated lipoplex
AS1	1.36	0.84
AS2	1.19	0.90
AS3	1.12	0.94
AS4	1.01	0.85

For DLS measurement and AFM observation, the change of size and shape of AS-ODN/CL complexes was slightly induced by the HHP treatment. For TEM observation, the lamellar structure of the non-treated AS-ODN/CL complexes was observed, whereas the amorphous structure including small and regular structure was observed for the HHP-treated AS-ODN/CL complexes. The hydrophobic microenvironment of AS-ODN/CL complexes was investigated by using fluorescent hydrophobic probes, such as Laurdan and pyrene. The fluorescent spectrum and intensity of these probes differed between AS-ODN/CL complexes with/without the HHP treatment. These results suggest that the structure of AS-ODN/CL complex was changed by the HHP treatment and then its change affect the efficiency of gene silencing.

Conclusions: We successfully improved the efficiency of gene suppression by using the HHP treated AS-ODN/CL complex. It was found that the change of the structure of AS-ODN/CL complex was induced by the HHP treatment. We believe that this change of lipoplex structure by the HHP treatment affected the efficiency of gene suppression. This HHP treatment for ON/CL complex appears to be a promising contribution to gene and ON delivery.

Acknowledgement: This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology. We would like to thank Group Director Hisatoshi Kobayashi and Dr. Keiji Kurashima of National Institute for Materials Science for their kind assistance and advice on TEM observation.

References:

[1] JB Opalinska, AM Gewirtz, *Nature Reviews Drug Discovery*, 2002, 1(7), 503-514. [2] T Kimura, H Konno, A Sano, T Fujisato, A Kishida, *Society For Biomaterials 2010 Annual meeting & exposition*, 901. [3] B Malaekeh-Nikouei, M Malaekeh-Nikouei, RK Oskuee, M Ramezani, *Nanomedicine*, 2009, 5(4), 457-462.

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