ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

A Novel Strategy for Selective Gene Delivery by Using the Inhibitory Effect of Blue Light on jetPRIME-Mediated Transfection

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ABSTRACT: Photodynamic control of gene delivery is a new technology with growing applications in gene therapy and basic cell research. Main approaches of light-selective gene delivery rely on the light-dependent enhancement of transfection efficiency. Studies focused on light-stimulated inhibitory regulation of transfection have rarely been reported. Here, we tried to establish a novel procedure of light-dependent inhibition of transfection. Our experiments, conducted with several types of commercial transfection reagents, revealed that jetPRIME-mediated transfection was strongly inhibited by blue light. Although the uptake of reagent–DNA complex was drastically reduced, preliminary exposure of cells or reagent–DNA complex to blue light had no inhibitory effect on the transfection efficiency. The inhibitory effect was wavelength-dependent and mediated by reactive oxygen species. Partial exposure of a culture vessel to blue light resulted in selective gene delivery into cells grown on the unexposed area of the vessel. By using this approach, different types of plasmid DNA were delivered into different areas in the culture vessel. This novel approach to the inhibitory control of transfection provides practical options for research and therapeutics.

Biotechnol. Bioeng. 2015;9999: 1–8.

KEYWORDS: selective gene delivery; blue light; jetPRIME

Introduction

Targeted gene delivery is important to achieve effective therapy with minimum side effect. Several therapeutic nanocarriers designed for specific targets were developed and contributed to this purpose (Peer et al., 2007). In addition, photodynamic control of DNA/RNA delivery became available and has growing potential applications in gene therapy and basic cell research. The majority of DNA/RNA–nanocarrier complexes enter cells by clathrin-mediated endocytosis. Trafficking of the complexes includes the following stages: cell binding, endocytosis, endosomal escape, and cytosolic transit (Medina-Kauwe et al., 2005). Endosomal escape, which avoids lysosomal degradation, is important for efficient polynucleotide delivery. Photodynamic induction of endosomal escape mediated by photosensitizer and light irradiation results in successful enhancement of transfection efficiency (Berg et al., 1999; Dougherty et al., 1998; Matsushita-Ishiodori and Ohtsuki, 2012; Nomoto et al., 2014; Oliveira et al., 2007, 2008; Selbo et al., 2010). However, studies focused on light-stimulated inhibitory regulation of transfection were rarely reported. Our experiments, conducted with several types of commercial transfection reagents, revealed that jetPRIME-mediated transfection was strongly inhibited by blue light irradiated from light-emitting diode (LED). Here we report a basic feature of the blue light-dependent inhibition of jetPRIME-mediated transfection and a novel procedure of photodynamic control of gene delivery by using the inhibitory effect.

Conflicts of interest: There are no conflicts of interest to declare.
Correspondence to: K. Takishima
Contract grant sponsor: Japanese Society for the Promotion of Science
Received 13 October 2015; Revision received 18 November 2015; Accepted 9 December 2015
Accepted manuscript online xx Month 2015;
Article first published online in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/bit.25906

Materials and Methods

Light Source and Irradiation Conditions

LED spot lights were used in the experiments to avoid rise in temperature of cells. 36-Blue LED Spotlight (RSD, Gangdong, China) was used for blue light irradiation. E27 9W LED Bulb RGB (Grandway Lighting Technology Co., Shenzhen, China) was used for green light irradiation. Intensity of the illumination was measured by Lux Meter LM-102 (MT Mother Tool, Ueda, Japan). The culture vessel was light irradiated from above. Intensity of illumination was adjusted by using an intensity controller-equipped light source or by adjusting the distance between the light source and the culture vessel. The spectrum of each light source was determined by using a Public Lab Foldable Mini-spectrometer (Public Lab, Cambridge, MA). The collected spectral data of the blue and green light were analyzed with ImageJ software (NIH, Bethesda, MD), without

uploading raw data to the Public Lab website. Spectral data of fluorescent light for calibration were collected and uploaded to the Public Lab website (http://spectralworkbench.org/capture) for calibration and the obtained scale of wavelength was applied to the spectra of blue and green light.

Cell Culture

HeLa, HEK293A, and HepG2 cells were maintained in DMEM (Gibco, ThermoFisher Scientific, Waltham, MA) supplemented with 10% fetal bovine serum (HyClone, GE Healthcare, Pittsburgh, PA) and penicillin–streptomycin (Wako, Osaka, Japan). These cells were sub-cultured every 3 days and kept at a density of less than 80% confluent.

Water Temperature in the Culture Vessel

Water temperature in the culture vessel was measured by an ND-X digital thermo-sensor (NISSO, Osaka, Japan). Measurements were taken at 2-h intervals for 12 h in the control and induction chambers with or without blue light irradiation.

Cell Viability Assay

Cell viability was determined by using the Cell Counting Kit-8 (Dojindo Molecular Technologies, Mashiki, Japan). Absorption at 450 nm was measured by Infinite M200 PRO (Tecan, Männedorf, Switzerland).

ROS Inhibition Assay

N-acetyl-L-cysteine (Sigma–Aldrich, St. Louis, MS) was dissolved in the buffer [20 mM HEPES (pH 7.0)] at concentration of 100 mM as a stock solution. *N*-acetyl-L-cysteine was administered to HeLa cells 1 h before transfection at final concentration of 2 and 4 mM. Cells were harvested 42 h after the transfection and transfection efficiency was determined.

Transfection

Cells were seeded at a density of 6×10^4 cells in each well of a 48-well culture plate and 12×10^6 cells in a 10-cm culture dish. Fourteen hours after seeding, the cells were transfected. The procedure of X-tremeGENE HP (Roche, Basel, Switzerland)-mediated transfection in 48-well culture plate was as follows. Plasmid DNA (120 ng) and 1.2 μL X-tremeGENE HP reagent were diluted with serum-free DMEM to a volume of 30 μL, and incubated for 15 min at 25°C before being added to each well. The procedure of Screen*F*ect™ A (Wako)-mediated transfection in 48-well culture plate was as follows. Plasmid DNA (120 ng) was diluted in 16 μL of buffer, then mixed with 16 μL of buffer containing 0.8 μL Screen*F*ect™ A, and incubated for 30 min at 25°C before being added to each well. The procedure of Lipofectamine LTX (Life Technologies)-mediated transfection in 48-well culture plate was as follows. Plasmid DNA (200 ng) and 0.7 μL Lipofectamine LTX were diluted with serum-free DMEM to a volume of 40 μL, and incubated for 25 min at 25°C before being added to each well. The procedure of jetPRIME (Polyplus Transfection, Illkirch, France)-mediated transfection in 48-well culture plate was as follows. Plasmid DNA (200 ng) and 0.4 μL jetPRIME reagent were mixed with 20 μL buffer, and incubated for 10 min at 25°C before being added to each well. The volume of the DNA-jetPRIME transfection solution applied to each well was reduced for lower dose of transfection such as 180 ng, 120 ng, 80 ng, and 40 ng plasmid DNA. Transfection in a 10-cm culture dish by jetPRIME was as follows. Plasmid DNA (4 μg) and 8 μL jetPRIME were mixed with 400 μL buffer and incubated for 10 min at 25°C. The incubated solution was diluted in culture medium to a volume of 10 mL and the mixture replaced the culture medium of the cells.

Plasmid DNA

pmCherry-C1, an mCherry-expressing vector composed of a cytomegalovirus immediate-early promoter, mCherry coding sequence, and SV40 polyadenylation signal, was kindly furnished by Dr. Akiyoshi Fukamizu. pCAG-GFP is a pCAGGS-based vector expressing green fluorescent protein (GFP). pCAGGS was established and kindly furnished by Dr. Jun-ichi Miyazaki (Niwa et al., 1991).

Transfection Efficiency

Forty-two hours after the transfection in blue light, DNA was extracted from the cells by using Isogen (Nippon Gene, Tokyo, Japan) reagent following DNA extraction procedure according to manufacturer's protocol. The extracted DNA was used in a quantitative Real-Time PCR to determine the quantity of the transfected pmCherry-C1 plasmid DNA, using the genomic β-actin gene as an internal control. DNA amplification was conducted by using the following primers. mCherry F; 5′-ttggacatcacctcccacaa-3′, mcherryR; 5′-tcggcgcgttcgtactgt-3′, β-actin F; 5′-tggatcagcaagcag-gagtatg-3′, β-actin R; 5′-gcatttgcggtggacgat-3′. DNA (30 ng) extracted from transfected cells was applied to Real-Time PCR. Real-Time PCR was conducted by using the Power SYBR Green PCR Master Mix (Life Technologies) and StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, MA) following the manufacturer's protocol. The quantity of pmCherry-C1 plasmid DNA, normalized by genomic β-actin gene, represented the transfection efficiency.

Irradiation of DNA–Transfection Reagents Complex before Transfection

DNA–Lipofectamine LTX mixed solution and DNA–jetPRIME mixed solution were prepared and applied separately to both exposed sample and shaded control. The exposed sample was incubated under 550 lux blue light for 1 h before the transfection. The shaded control was incubated in dark for 1 h before the transfection.

Quantification of mRNA Expression

Forty-two hours after transfection in blue light, RNA was extracted from the cells by using Isogen reagents following RNA extraction

procedure of manufacturer's protocol. RNA samples were used in reverse transcription by Primescript™ RT Reagent Kit (TaKaRa, Kusatsu, Japan), following the manufacturer's protocol. Diluted solution (×20) of the reverse transcription product was applied to Real-Time PCR. Real-Time PCR was conducted to determine the expression of mCherry and β-actin mRNAs, by using Power SYBR Green PCR Master Mix and the primers described above. The mCherry expression was normalized by β-actin expression.

Quantification of Remaining Plasmid DNA in Culture Media of Transfected Cells

Forty-two hours after transfection, the culture media of the transfected cells was collected and the remaining plasmid DNA in the culture media was extracted by using the QIAamp DNA Mini Kit (QIAGEN, Venlo, the Netherlands) following the manufacturer's protocol, and quantified by Real-Time PCR, as described above.

Partial Shading of Culture Vessel in Blue Light Irradiation

The shade was made of black paper and designed to cover half of a 10-cm culture dish with a gap for aeration.

Counting Cells With Strong Fluorescence in a Culture Dish

Forty-eight hours after half-shaded transfection in blue light, cells were fixed in 4% paraformaldehyde/phosphate buffered saline (PBS) for 1 h. Fixed cells were washed twicw in PBS and stained with 5 μg/mL DAPI/PBS solution. Fluorescent microscopic images were obtained by using ECLIPSE TE2000-E (Nikon, Tokyo, Japan) with objective lens ELWD 20X/0.45 (Nikon) and digital camera ORCA-ER C4724-95 (Hamamatsu Photonics K.K., Hamamatsu, Japan). NIS-Elements Basic Research software version 4.20 was used for obtaining and processing the microscopic images. Exposure time was 300 ms for mCherry and GFP signals, and 400 ms for DAPI. Counting of cells with strong fluorescence was conducted with ImageJ software. Thresholds for mCherry and GFP signals were set to 27–255 and 50–255, respectively.

Statistical Analysis

Student's *t*-test was performed for statistical analysis of data. Significance level to reject the null hypotheses was set to 0.05.

Results

Screening of Light-Reactive Transfection Reagents

To consider a prospective application of two-photon excitation in vivo, we assayed the blue light reactivity of several commercial transfection reagents, such as X-tremeGENE HP, Screen*Fect*™ A, Lipofectamine LTX, and jetPRIME. The transfection efficiency of each reagent in blue light irradiation was determined. Blue light irradiation from LED in the incubator had no effect on water temperature in the culture vessel, or the survival rate of HeLa cells in the vessel (Fig. 1A–C). The transfection efficiency with

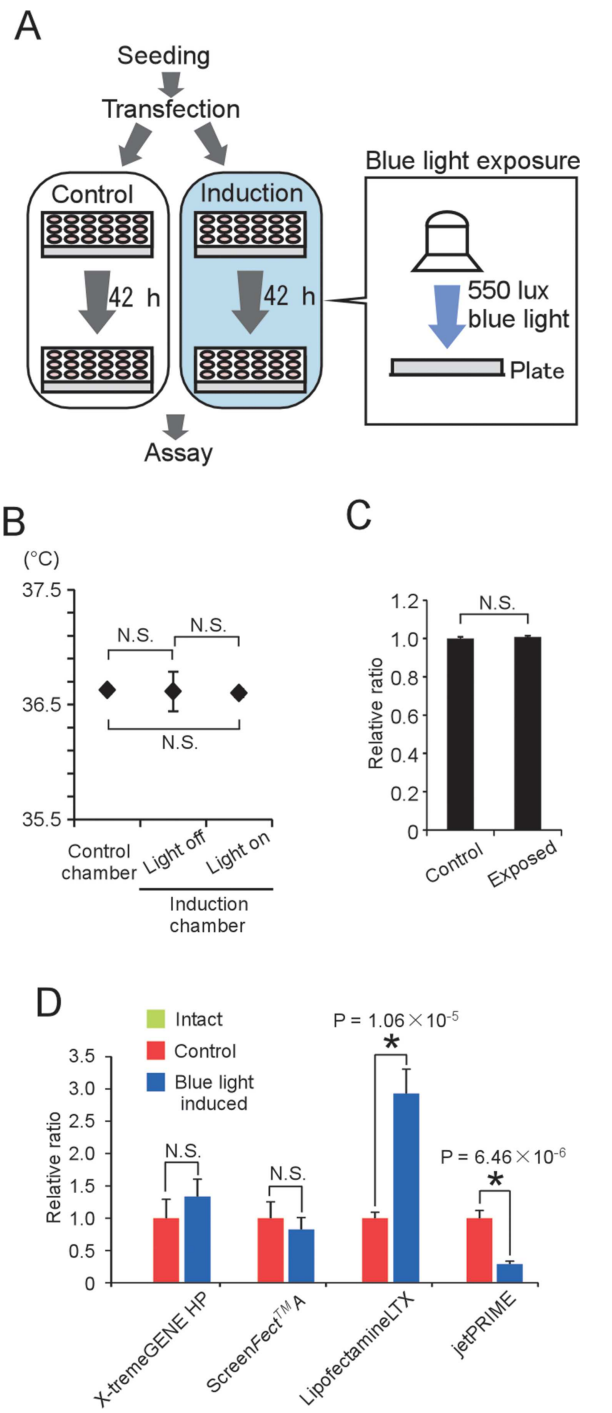

Figure 1. Screening of light-reactive transfection reagents. (**A**) Procedure of transfection under blue light irradiation. (**B**) Temperature measurements in control chamber and induction chamber with or without blue light irradiation. (**C**) Viability of cells incubated in control and blue light-irradiated induction chamber. Cells were plated at density of 2×10^4 per well in a 48-well culture plate, and cultured in the chambers for 24 h. Relative ratio of cell viability determined by WST-8 assay is shown. $N = 20$. (**D**) Relative ratio of efficiency of transfection mediated by transfection reagents in the control and induction chambers. Sample number of each experiment is as follows. X-tremeGENE HP, Intact = 4, Control = 10, Induced = 10; Screen*Fect*™ A, Intact = 4, Control = 10, Induced = 10; Lipofectamine LTX, Intact = 3, Control = 20, Induced = 20; jetPRIME, Intact = 4, Control = 20, Induced = 20. Error bars: S.E. *P*-values are shown in the graph with significant difference. N.S., no significant difference.

Lipofectamine LTX reagent was drastically increased in 550 lux blue light (Fig. 1D). On the contrary, the transfection efficiency with jetPRIME was strongly reduced by blue light irradiation (Fig. 1D). The transfection efficiencies of X-tremeGENE HP and Screen*Fect*^{TM} A were not affected by blue light (Fig. 1D). Enhancement in the Lipofectamine LTX-mediated transfection by blue light was observed only in HeLa cells and not in HEK293A and HepG2 cells (Fig. 2A). To study the mechanism of the enhancement, DNA–Lipofectamine LTX complex was preliminary exposed to blue light before transfection. The transfection efficiency was increased by the preliminary exposure (Fig. 2B).

Blue Light-Dependent Inhibition of jetPRIME-Mediated Trasnfectin

Blue light-induced inhibition of jetPRIME-mediated transfection was observed in HeLa, HEK293A, and HepG2 cells (Figs. 1D and

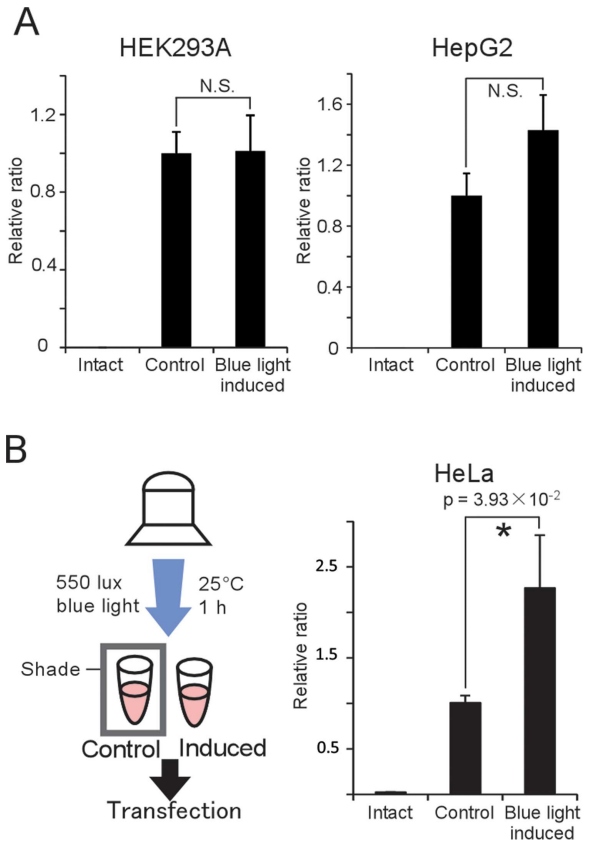

Figure 2. Blue light-dependent enhancement of Lipofectamine LTX-mediated transfection. (**A**) Cell type specificity of blue light-dependent enhancement of Lipofectamine LTX-mediated transfection. Sample numbers of the experiment are as follows. Intact = 3, Control = 10, Induced = 10 in HEK293A; Intact = 4, Control = 10, Induced = 10 in HepG2. (**B**) Preliminary exposure of DNA–Lipofectamine LTX complex to blue light before the transfection into HeLa cells. Scheme of the experimental procedure (left) and transfection efficiency of control and preliminary exposed samples (right) is shown. Sample numbers of the experiment are as follows. Intact = 3, Control = 19, Induced = 20. Error bars: S.E. *P*-values are shown in the graph with significant difference. N.S., no significant difference.

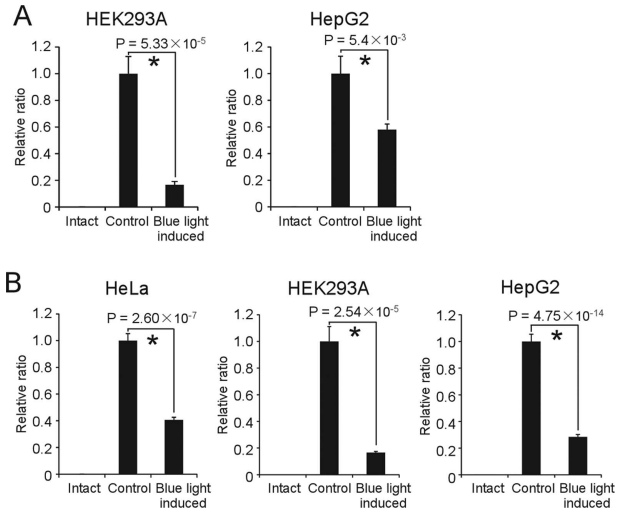

Figure 3. Cell type specificity of blue light-dependent inhibition of jetPRIME-mediated transfection. (**A**) Relative ratio of transfection efficiency of control and induced samples in HEK293A and HepG2 cells. Sample numbers of the experiment are as follows. Intact = 3, Control = 10, Induced = 10 in HEK293A; Intact = 3, Control = 18, Induced = 17 in HepG2. (**B**) Relative ratio of mCherry mRNA expression of control and induced samples in HeLa, HEK293A, and HepG2 cells. Sample numbers of the experiment are as follows. Intact = 4, Control = 10, Induced = 10 in HeLa; Intact = 4, Control = 10, Induced = 10 in HEK293A; Intact = 3, Control = 18, Induced = 17 in HepG2. Error bars: S.E. *P*-values are shown in the graph with significant difference. N.S., no significant difference.

3A). Associated with the decrease in the transfection efficiency under blue light, expression of the reporter gene mRNA derived from transfected plasmid DNA was significantly decreased in the cells exposed to blue light (Fig. 3B). The extent of blue light-induced inhibition of jetPRIME-mediated transfection was different, depending on the cell types. Stronger and weaker inhibitions, compared to that in HeLa cells, were observed in HEK293A and HepG2 cells, respectively. Remaining plasmid DNA in the culture media of transfected HeLa cells was analyzed by using relative quantitative PCR. The quantity was seven times higher in blue light-exposed samples, compared to that in the control (Fig. 4A). We next investigated whether blue light irradiation modifies reactivity or formation rate of DNA–jetPRIME complex. Preliminary exposure of DNA–jetPRIME complex before transfection had no adverse effect on the transfection efficiency (Fig. 4B). We further investigated the possibility that blue light irradiation alters cell receptivity to the transfection complex. Fourteen hours of blue light irradiation to HeLa cells before transfection had no significant effect on the transfection efficiency (Fig. 4C). To confirm the wavelength specificity of the transfection inhibition, we examined whether green light has an inhibitory effect on jetPRIME-mediated transfection. Green light at an intensity of 550 lux had no adverse effect on the transfection (Fig. 4D).

Blue light irradiation stimulates ROS production in cultured cells (Lan et al., 2015). Previous report revealed that inhibition of ROS production by an antioxidant dramatically improved the transfection efficiency mediated by amphiphilic copolymers based on poly-ethylenimine and poly (D,L-lactide-co-glycolide) (Lee et al., 2013).

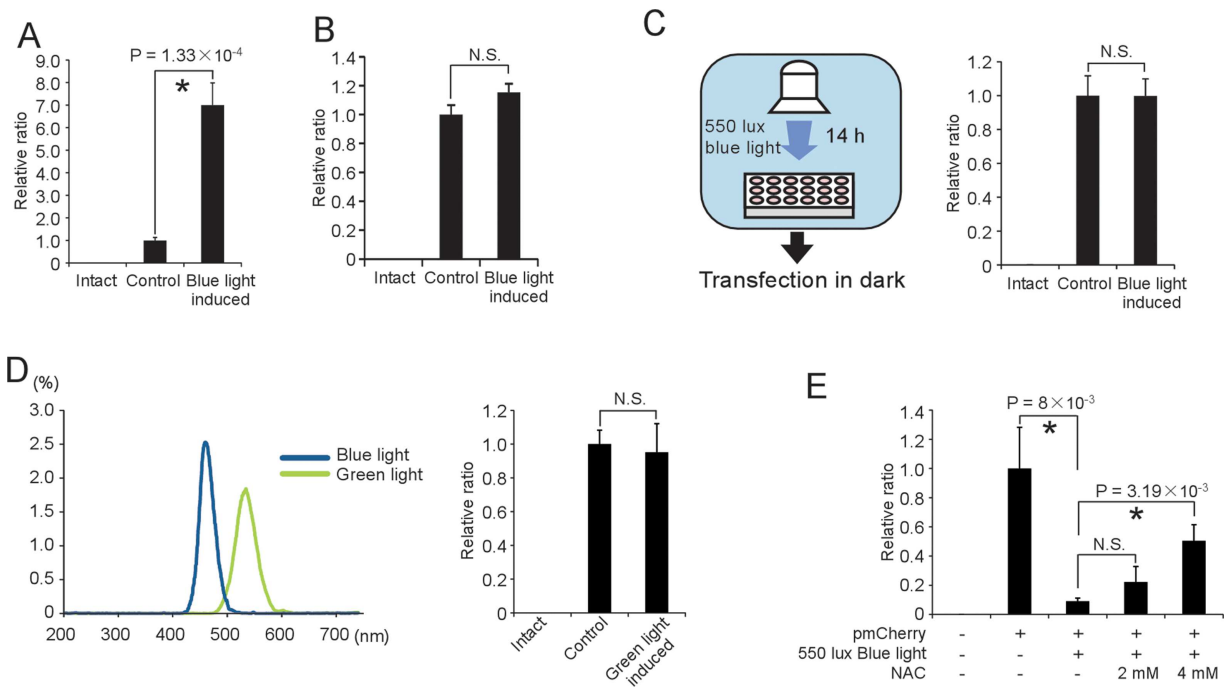

Figure 4. Wavelength-dependent inhibition of DNA–jetPRIME complex uptake. (**A**) Relative ratio of remaining mCherry-C1 plasmid DNA in the culture media of control and induced cells. Sample numbers of the experiment are as follows. Intact = 4, Control = 10, Induced = 10. (**B**) Preliminary exposure of DNA–jetPRIME complex to blue light before the transfection. Relative ratio of transfection efficiency of control and preliminary exposed samples are shown. Sample numbers of the experiment are as follows. Intact = 3, Control = 10, Induced = 10. The procedure is same as described in Figure 2B. (**C**) Preliminary exposure of HeLa cells to blue light before the transfection. Scheme of the experimental procedure (left) and relative ratio of transfection efficiency of control and preliminarily exposed samples (right) are shown. Samples were collected 42 h after the transfection and assayed. Sample numbers of the experiment are as follows. Intact = 4, Control = 10, Induced = 10. (**D**) Effect of green light irradiation on jetPRIME-mediated transfection. Spectrum of blue and green light used in the experiments (left) and relative ratio of transfection efficiency of control and green light irradiated samples (right) are shown. Sample numbers of the experiment are as follows. Intact = 4, Control = 10, Induced = 10. (**E**) *N*-acetyl-L-cysteine (NAC) administration to cells transfected under 550 lux blue light. Transfection efficiency of each condition is shown. Sample number of each treatment group excepting intact = 10. Intact = 4. Error bars: S.E. *P*-values are shown in the graph with significant difference. N.S., no significant difference.

To confirm involvement of ROS production in the inhibitory effect of blue light on jetPRIME-mediated transfection, an antioxidant, *N*-acetyl-L-cysteine, was administered to cells transfected under blue light irradiation. *N*-acetyl-L-cysteine administration significantly improved the transfection efficiency under blue light (Fig. 4E).

Selective Gene Delivery in a Specific Area on a Culture Vessel by Partial Shading

Modification of jetPRIME-mediated transfection by blue light is applicable to wider variety of cell types compared to that of Lipofectamine LTX-mediated transfection. We applied the inhibition of jetPRIME-mediated transfection to selective gene delivery in a culture vessel. Half of a 10-cm culture dish was covered by a black-colored shade and exposed to 1,800 lux blue light (Fig. 5A). For this large-scale experiment, conditions of transfection were modified to reduce cell damage. Several experimental conditions were tested in 48-well culture plate. Transfection with 40 ng DNA-0.08 μL jetPRIME in the 48-well culture plate was less cytotoxic and had minimum necessary level of transfection efficiency in HeLa cells (Fig. 5B). The experimental condition was scaled up and applied to HeLa cells cultured in a half-shaded 10-cm culture dish in blue light. Cells with strong mCherry reporter fluorescence were counted in the half-shaded 10-cm culture dish. Counts in grids on the shaded and exposed sides were calculated in percentage. The transfected cell counts were significantly higher in the grids on the shaded side, compared to the counts in the grids on the exposed side (Fig. 5C). Even neighboring grids, encompassing the shaded-exposed boundary, displayed significant difference in transfected cell counts (Fig. 5C). The selective gene delivery by using the inhibitory effect of blue light irradiation on jetPRIME-mediated transfection was also confirmed with different plasmid DNA, pCAG-GFP. Essentially, similar result was observed for the GFP signal in pCAG-GFP transfection (Supplemental Fig. S1).

By using this strategy, two different reporter-expressing plasmid DNAs, pmCherry-C1 and pCAG-GFP, were delivered to different areas in a 10-cm culture dish. pmCherry-C1 was transfected into HeLa cells in 10-cm culture dish, and side A was shaded under 3,000 lux blue light irradiation for 12 h. Following medium replacement, pCAG-GFP was transfected into the cells in the same dish, and side B was shaded under 3,000 lux blue light irradiation for 12 h (Fig. 6A). After the second transfection under light exposure was completed, the medium was replaced and the cells were incubated for 12 h before being fixed and stained for counting (Fig. 6A). Microscopic observation revealed dominant mCherry and GFP expression in side A and side B, respectively, in the culture dish

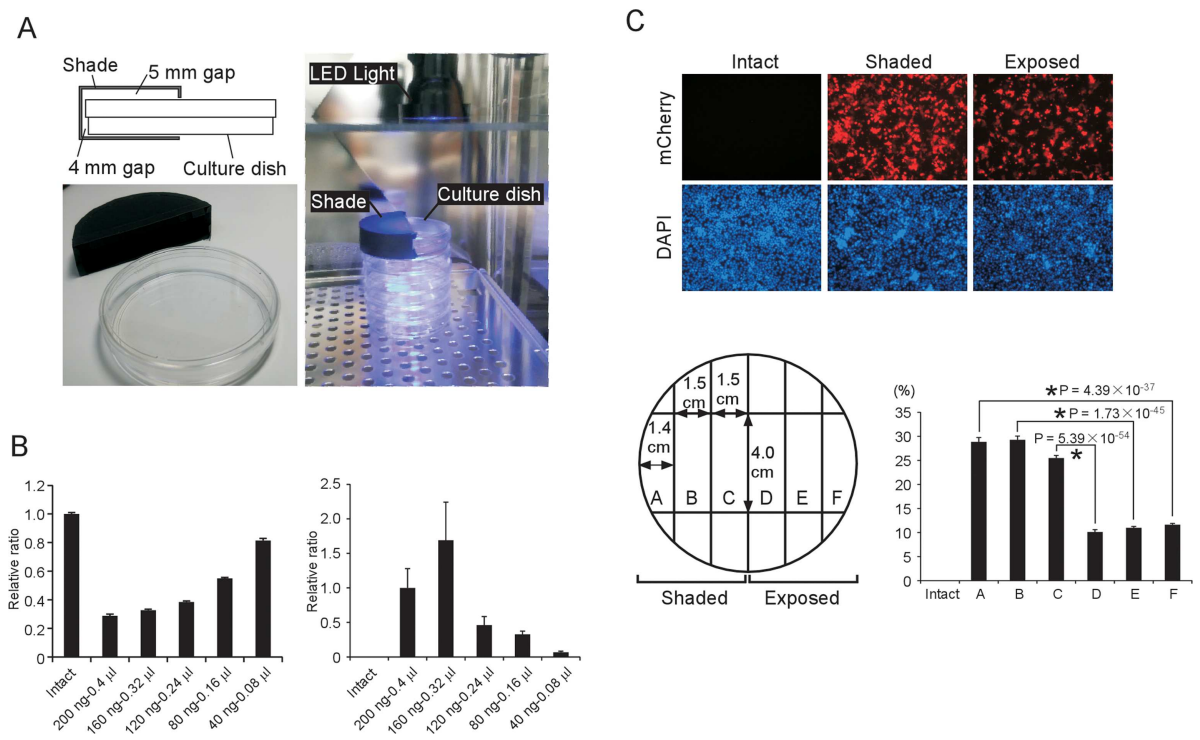

Figure 5. Selective gene delivery in a culture vessel by partial shading. (**A**) Scheme of partial shading. Schematic drawing and photograph of a half shade used in the experiment (left) and photograph of partial shading of culture dish in blue light irradiation are shown (right). (**B**) Dose-dependent manner of cell viability and transfection efficiency. Cell viability (left) and transfection efficiency (right) in each dose of DNA–jetRPIME are shown in graphs; $N = 6$. (**C**) Selective gene delivery by partial shading. Representative photographs of fluorescent microscopic images in intact, shaded, and exposed areas are shown (upper panel). Grid setting on a culture dish (lower left) and counts of cells with strong mCherry fluorescence in the grids are shown. Sample numbers of the experiment are as follows. Intact = 10, grids A, B, C, D, E, F = 100. Error bars: S.E. *P*-values are shown in the graph with significant difference.

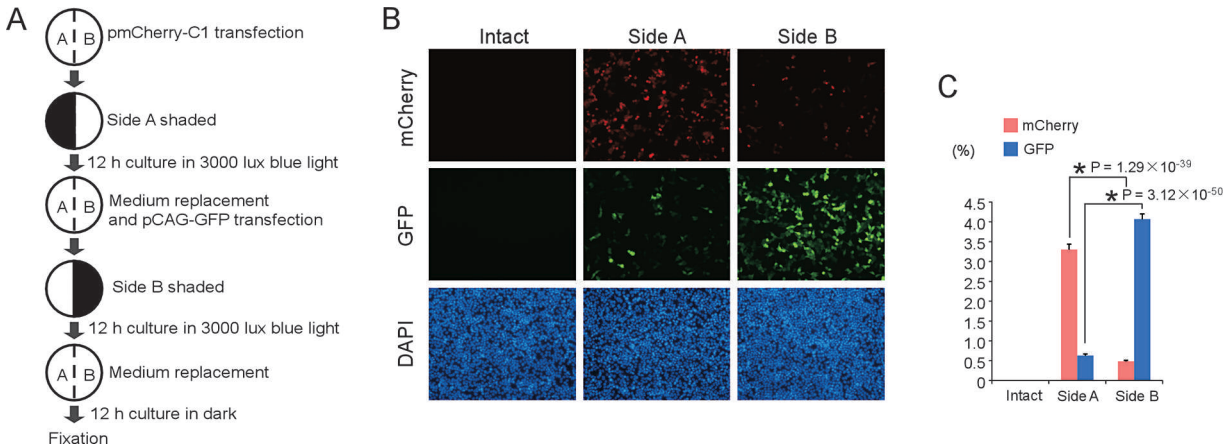

Figure 6. Delivery of two different plasmid DNAs in specific areas of a culture dish. (**A**) Scheme of experimental procedure. (**B**) Representative photographs of fluorescent microscopic images in intact, Sides A and B are shown. (**C**) Counts of cells with strong mCherry and GFP fluorescence in each side are shown. The cell count data were collected from grids located in the center of each side that were equivalent to the grids B or E described in Figure 5C. Sample numbers of the experiment are as follows. Intact = 10, Side A = 100, Side B = 100. Error bars: S.E. *P*-values are shown in the graph with significant differences.

(Fig. 6B). The total fluorescence signal was weaker than that observed in longer transfection time. Greater difference in the counts of cells with strong mCherry fluorescence in each side was observed under more intense blue light irradiation (Fig. 6B and C). The number of cells with strong mCherry fluorescence was approximately seven times higher in the shaded area. Essentially, similar results were observed for the GFP signal in pCAG-GFP transfection (Fig. 6B and C).

Discussion

We evaluated the transfection efficiencies of commercially available transfection reagents under blue light irradiation and found that the efficiencies of both Lipofectamine LTX- and jetPRIME-mediated transfection were affected. The efficiency of Lipofectamine LTX-mediated transfection was increased by preliminary blue light irradiation of the transfection reagent–DNA complex, suggesting that blue light accelerates the complex formation or increases the binding reactivity of the transfection complex for cell membrane. Among the reagents examined, Screen*Fect*™ A and Lipofectamine LTX are liposomal transfection reagents. The absence of blue light-dependent enhancement of Screen*Fect*™ A-mediated transfection suggests that the blue light-stimulated enhancement is unlikely caused by cationic liposome structure itself. In contrast, jetPRIME, a cationic polymer-based reagent, gave rise to no detectable change in the transfection efficiency when the reagent–DNA complex was exposed before transfection to blue light. Moreover, blue light irradiation on cells after jetPRIME-mediated transfection lead to decrease in transfection efficiency, and this inhibitory effect was significantly diminished by administration of *N*-acetyl-L-cysteine, presumably due to the reduction of ROS levels. Although molecular structure and constitution of jetPRIME are unknown, pore formation and proton sponge mechanism might be involved in the process of jetPRIME-mediated cytosolic transition of cargo DNA. Peptide-based pore-forming agents undergo conformational change in acidic condition of early endosome and make pore on the endosomal membrane facilitating cytosolic release of entrapped DNA from the vesicle (Medina-Kauwe et al., 2005). Function of such peptide-based pore-forming agents might be affected by oxidative damage attributed to cellular ROS activity induced by blue light. Proton sponge property of transfection reagents lead excessive endosomal H^+ uptake via ATP-dependent proton pump and following Cl^- and H_2O influx, causing endosomal rupture and cargo DNA release (Medina-Kauwe et al., 2005). Activity of ATP-dependent proton pump, vacuolar H(+)-ATPase, is inhibited by hydrogen peroxide (Brisseau et al., 1994). If the cytosolic transition of DNA from endosome largely depends on proton sponge mechanism for jetPRIME-mediated transfection, the process can be inhibited by ROS induced by blue light. Amount of cellular ROS induced by blue light is different, depending on the cell type (Lan et al., 2015). This fact could account for our result that the difference in blue light inhibitory effect depended on the cell-type. Half-life of ROS, such as $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet -}$ is less than 10^{-5} sec (Giorgio et al., 2007). This suggests that preliminary exposure of HeLa cells to blue light before transfection has no inhibitory effect on jetPRIME-mediated transfection because of the short half-life of ROS induced by blue light.

In this study, we demonstrated that blue light-induced inhibitory approach using jetPRIME transfection reagent is applicable to selective gene delivery. Photochemical internalization, based on a photosensitizer that chemically destabilizes endosomal membrane after illumination, is a powerful tool for the enhancement of gene delivery, but still this approach brings about undesirable gene delivery to untargeted area (Bøe et al., 2007, 2008). Aluminum phthalocyanine disulfonate ($AlPcS_{2a}$) is one of the most useful photosensitizer, which is stimulated by red light (670 nm) irradiation. Transfection of jetPRIME-DNA complex with $AlPcS_{2a}$ under spatially selective irradiation of blue and red lights may enable marked region-specific gene expression. Thus, combined use of wavelength-dependent inhibitory and enhancing strategy may provide a new powerful approach to gene delivery. Enhancing strategy mediated by red light irradiation and $AlPcS_{2a}$ can provide 13–22-fold increase in transfection efficiency depending on cell types (Prasmickaite et al., 2004). By using the blue light-induced inhibitory approach, transfection efficiency can be reduced to 14.6% of that of the control (Fig. 6C). If these enhancing and inhibitory approaches can be combined successfully in a culture vessel, more than 80-fold higher transfection efficiency might be obtained in targeted area comparing to that of untargeted area. Although the precise molecular or quantum mechanism for the inhibition of transfection efficiency is still unclear, this novel strategy has the potential to provide a practical and simple option for spatiotemporal control of gene delivery.

This work was partly supported by grants from Japanese Society for the Promotion of Science. We thank Dr. Akiyoshi Fukamizu for technical advice and for providing experimental materials and Dr. Jun-ichi Miyazaki for providing experimental materials.

References

Berg K, Selbo PK, Prasmickaite L, Tjelle TE, Sandvig K, Moan J, Gaudernack G, Fodstad Ø, Kjølsrud S, Anholt H, Rodal GH, Rodal SK, Høgset A. 1999. Photochemical internalization: A novel technology for delivery of macromolecules into cytosol. Cancer Res 59:1180–1183.

Bøe S, Longva AS, Hovig E. 2007. Photochemically induced gene silencing using small interfering RNA molecules in combination with lipid carriers. Oligonucleotides 17:166–173.

Boe S, Longva AS, Hovig E. 2008. Evaluation of various polyethylenimine formulations for light-controlled gene silencing using small interfering RNA molecules. Oligonucleotides 18:123–132.

Brisseau GF, Tsai O, Nordström T, Marshall JC, Grinstein S, Rotstein OD. 1994. Oxidant stress inhibits pH regulatory mechanisms in murine peritoneal macrophages. Surgery 116:268–274; discussion 274–275.

Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J, Peng Q. 1998. Photodynamic therapy. J Natl Cancer Inst 90:889–905.

Giorgio M, Trinei M, Migliaccio E, Pelicci PG. 2007. Hydrogen peroxide: A metabolic by-product or a common mediator of ageing signals. Nat Rev Mol Cell Biol 8:722–728.

Lan CC, Lu EY, Pan HJ, Lee CH. 2015. Directional migration of cancer cells induced by a blue light intensity gradient. Biomed Opt Express 6:2624–2632.

Lee MS, Kim NW, Lee K, Kim H, Jeong JH. 2013. Enhanced transfection by antioxidative polymeric gene carrier that reduces polyplex-mediated cellular oxidative stress. Pharm Res 30:1642–1651.

Matsushita-Ishiodori Y, Ohtsuki T. 2012. Photoinduced RNA interference. Acc Chem Res 45:1039–1047.

Medina-Kauwe LK, Xie J, Hamm-Alvarez S. 2005. Intracellular trafficking of nonviral vectors. Gene Ther 12:1734–1751.

Niwa H, Yamamura K, Miyazaki J. 1991. Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108:193–199.

Nomoto T, Fukushima S, Kumagai M, Machitani K, Arnida, Matsumoto Y, Oba M, Miyata K, Osada K, Nishiyama N, Kataoka K. 2014. Three-layered polyplex micelle as a multifunctional nanocarrier platform for light-induced systemic gene transfer. Nat Commun 5:3545.

Oliveira S, Fretz MM, Høgset A, Storm G, Schiffelers RM. 2007. Photochemical internalization enhances silencing of epidermal growth factor receptor through improved endosomal escape of siRNA. Biochim Biophys Acta 1768:1211–1217.

Oliveira S, Høgset A, Storm G, Schiffelers RM. 2008. Delivery of siRNA to the target cell cytoplasm: Photochemical internalization facilitates endosomal escape and improves silencing efficiency, in vitro and in vivo. Curr Pharm Des 14:3686–3697.

Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. 2007. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2:751–760.

Prasmickaite L, Høgset A, Olsen VM, Kaalhus O, Mikalsen S-O, Berg K. 2004. Photochemically enhanced gene transfection increases the cytotoxicity of the herpes simplex virus thymidine kinase gene combined with ganciclovir. Cancer Gene Ther 11:514–523.

Selbo PK, Weyergang A, Høgset A, Norum OJ, Berstad MB, Vikdal M, Berg K. 2010. Photochemical internalization provides time- and space-controlled endolysosomal escape of therapeutic molecules. J Control Release 148:2–12.

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