doi:10.1246/cl.2011.298

Published on the web February 16, 2011

Ultrasound-mediated Transfection with Liposomal Bubbles Delivers Plasmid DNA Directly into Nucleus

Takeshi Kawazu,[1] Kazumi Hakamada,[2] Yusuke Oda,[3] Jun Miyake,[2] Kazuo Maruyama,[3] and Takeshi Nagasaki*[1]
[1]*Department of Applied Chemistry and Bioengineering, Graduate School of Engineering, Osaka City University, 3-3-138 Sumiyoshi-ku, Osaka 558-8585*
[2]*Department of Mechanical Science and Bioengineering, Graduate School of Engineering Science, Osaka University, 1-3 Machikaneyama, Toyonaka, Osaka 560-8531*
[3]*Department of Biopharmaceutics, School of Pharmaceutical Sciences, Teikyo University, 1091-1 Suwarashi, Sagamiko, Sagamihara, Kanagawa 229-0195*

(Received January 5, 2011; CL-110002; E-mail: nagasaki@bioa.eng.osaka-cu.ac.jp)

Transfection using ultrasound exposure in the presence of nanobubbles can overcome an important barrier for nonviral gene delivery, that is entering the nucleus without cell division. The monitoring of the relationship between fluorescent protein expression and cell division reveals that ultrasound-mediated transfection with liposomal bubbles is independent on the disappearance of nuclear membrane at mitosis.

In many cases, nonviral gene transfer depends on the cell cycle.[1] Giorgio et al. found that mitosis enhances transgene expression of plasmid DNA delivered by cationic liposomes.[2] The critical rate-determining step for nonviral gene delivery is nuclear entry of an exogenous gene.[3] Although enormous efforts have been directed to this problem, an estimation method has not been established for a long time.[4] Recently Hakamada, Miyake, and co-worker reported the examination of cell morphology and the dynamics of EGFP gene expression by using a fluorescent microscopic apparatus capable of monitoring single cell behavior.[5] With a lipoplex (Lipofectamine LTX, Invitrogen, Carlsbad, CA, USA) as nonviral vector, their results definitely showed that the onset timing of gene expression depended on cell division. This work semiquantitatively demonstrates for the first time that the nuclear envelope determines the rate of nonviral gene delivery.

On the other hand, it is well known that physical methods such as electroporation and sonoporation are effective gene-delivery methods especially for primary cells.[6] Wagner et al. reported that gene transfer by electroporation shows hardly any cell cycle dependence.[7] Maruyama et al. succeeded in the enhancement of sonoporation transfection efficiency by combining ultrasound and acoustic liposomes (bubble liposomes) which contain the ultrasound imaging gas perfluoropropane.[8] Bubble liposomes could act as an effective gene-delivery tool not only in vitro but also in vivo.[9] Under ultrasound exposure, the cavitation of nanobubbles in liposomes induced mechanical constraints on the plasma membrane and increased the membrane permeability by the creation of nanosize pores. The kinetics of protein expression was significantly faster for sonoporation than for lipofection that requires endocytosis.[10] However, very little is known whether ultrasound affects plasmid entry into the nucleus. In this paper, cell division and protein expression kinetics on ultrasound-mediated gene delivery with bubble liposomes have been studied by Hakamada's method.[5]

In 24-well culture plate, NIH3T3 cells (mouse fibroblast) were seeded at 6×10^4 cells per well and cultured at 37 °C with 5% CO_2 atmosphere in 10% FBS-containing DMEM. Cells were transfected with pCMV-Venus encoding a green fluorescent protein (venus) under the control of the CMV promoter. After transfection, phase-contrast and fluorescent images of cells in each well were recorded at intervals of 10 min for 30 h, and their exposure times were 50 and 400 ms, respectively. The measurement was started at 1 h post-transfection. As a compared nonviral gene carrier, linear poly(ethyleneimine)s (jetPEI, Polyplus-Transfection, Illkirch, France) was used because it showed high transfection ability in vitro and in vivo.[11] The transfection procedure was followed with manufacturer's instruction. On the other hand, preparation of bubble liposomes and transfection of plasmid DNA using bubble liposomes are carried out according to similar way previously reported by Maruyama et al.[8b] The ultrasound was exposed for 10 s under following conditions: frequency, 2 MHz, duty, 50%; intensity, 2.5 W cm^{-2}; burst rate, 2 Hz using Sonopore 3000 (NEPA GENE, CO., LTD., Chiba, Japan).

The results of jetPEI (Figure 1a) show a similar relationship between the timing of cell division and the onset timing of gene expression as that by lipofection.[5] The peak of protein expressing cells appeared around 10 h post-transfection

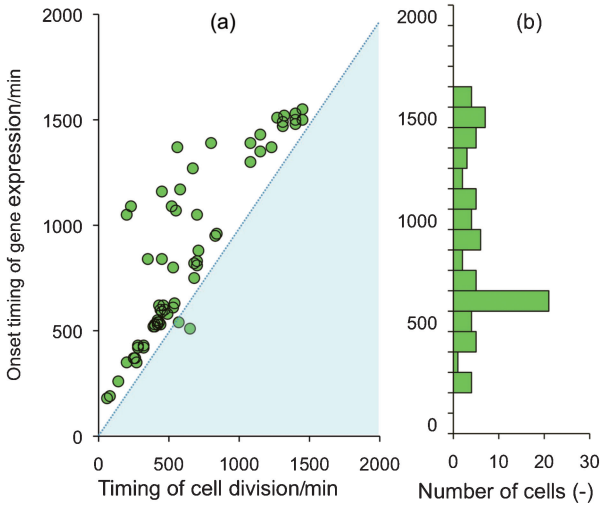

Figure 1. (a) Correlation between the timing of cell division and the onset of protein expression by jetPEI-mediated transfection. (b) Distribution of the cell number on the onset timing on jetPEI-mediated transfection.

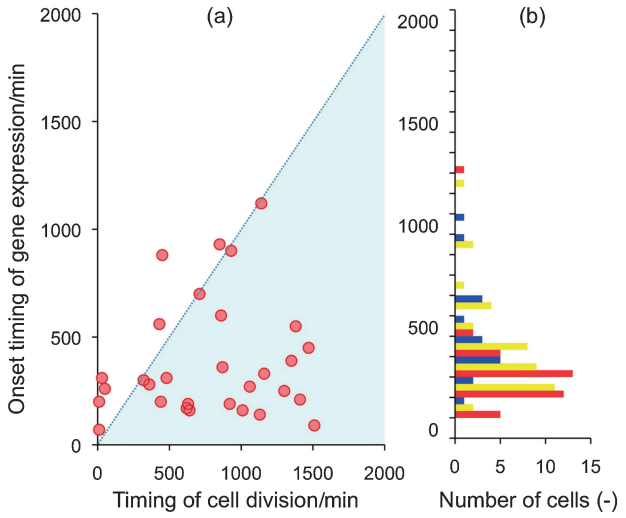

Figure 2. (a) Correlation between the timing of cell division and the onset of protein expression on ultrasound-mediated transfection with bubble liposomes. (b) Distribution of the cell number on the onset timing on ultrasound-mediated transfection with bubble liposomes; expressing after division (blue), expressing before division (yellow), expressing without division (red), respectively.

(Figure 1b). The polymeric nonviral gene transfection obviously depended on cell division although Wagner et al. mentioned cell-cycle-independent gene transfection by linear PEI.[7]

On the contrary, correlation between the timing of cell division and the onset of protein expression by ultrasound-mediated transfection in the presence of liposomal bubbles is shown in Figure 2. Under these conditions, sonoporation did not show cytotoxicity at all by WST assay. There is no relationship between the timing of cell division and the onset of protein expression. Before 10 h post-transfection, more than 80% of cells expressed the protein without cell division unlike jet-PEI (Figure 2b). The kinetics of gene expression showed that ultrasound-mediated transfection with nanobubbles allowed a rapid and direct transfer of naked DNA not only into the cytoplasm but also into the nucleus, probably via ultrasound-induced pores in the nuclear membrane. The transient perforation of plasma membrane by collapse of microbubbles was confirmed by SEM observation by Kudo et al.[12] The maximum velocity of the microstreams may be 700 m s^{-1}.[13] These rapid and strong microstreams could induce transient pores at plasma and nuclear membranes simultaneously and enhance the permeability of exogenous DNA into the nucleus. The expression after cell division showed an earlier time profile than that of jet-PEI; that also suggests direct transfer of plasmid DNA into cytoplasm rather than endocytosis.

In summary, our results demonstrate that ultrasound-mediated gene transfer with liposomal bubbles is nontoxic and effective under appropriate conditions even toward nondividing cells. We expect these findings will enhance the utility of current sonoporation, particularly in gene and drug delivery.

References

1 S. Brunner, T. Sauer, S. Carotta, M. Cotton, M. Saltik, E. Wagner, *Gene Ther.* **2000**, *7*, 401.
2 W. C. Tseng, F. R. Haselton, T. D. Giorgio, *Biochim. Biophys. Acta, Gene Struct. Expression* **1999**, *1445*, 53.
3 M. E. Dowty, P. Williams, G. Zhang, J. E. Hagstrom, J. A. Wolff, *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 4572; J. J. Ludtke, M. G. Sebestyen, A. Wolff, *Mol. Ther.* **2002**, *5*, 579; C. W. Pouton, K. M. Wagstaff, D. M. Roth, G. W. Mosley, D. A. Jans, *Adv. Drug Delivery Rev.* **2007**, *59*, 698.
4 T. Nagasaki, T. Kawazu, T. Tachibana, S. Tamagaki, S. Shinkai, *J. Controlled Release* **2005**, *103*, 199; T. Masuda, H. Akita, T. Nishio, K. Niikura, K. Kogure, K. Ijiro, H. Harashima, *Biomaterials* **2008**, *29*, 709; A. P. Lam, D. A. Dean, *Gene Ther.* **2010**, *17*, 439.
5 K. Hakamada, S. Fujita, J. Miyake, *J. Biosci. Bioeng.* **2010**, *109*, 62.
6 M. Duvshani-Eshet, L. Baruch, E. Kesselman, E. Shimoni, M. Machluf, *Gene Ther.* **2006**, *13*, 163; J. M. Escoffre, J. Teissié, M. P. Rols, *J. Membr. Biol.* **2010**, *236*, 61.
7 S. Brunner, E. Fürtbauer, T. Sauer, M. Kursa, E. Wagner, *Mol. Ther.* **2002**, *5*, 80.
8 a) R. Suzuki, T. Takizawa, Y. Negishi, N. Utoguchi, K. Maruyama, *J. Drug Targeting* **2007**, *15*, 531. b) R. Suzuki, T. Takizawa, Y. Negishi, K. Hagisawa, K. Tanaka, K. Sawamura, N. Utoguchi, T. Nishioka, K. Maruyama, *J. Controlled Release* **2007**, *117*, 130.
9 R. Suzuki, T. Takizawa, Y. Negishi, N. Utoguchi, K. Sawamura, K. Tanaka, E. Namai, Y. Oda, Y. Matsumura, K. Maruyama, *J. Controlled Release* **2008**, *125*, 137; R. Suzuki, E. Namai, Y. Oda, N. Nishiie, S. Otake, R. Koshima, K. Hirata, Y. Taira, N. Utoguchi, Y. Negishi, S. Nakagawa, K. Maruyama, *J. Controlled Release* **2010**, *142*, 245.
10 S. Mehier-Humbert, T. Bettinger, F. Yan, R. H. Guy, *J. Controlled Release* **2005**, *104*, 203.
11 O. Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix, J. P. Behr, *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7297.
12 N. Kudo, K. Okada, K. Yamamoto, *Biophys. J.* **2009**, *96*, 4866.
13 S. Hernot, A. L. Klibanov, *Adv. Drug Delivery Rev.* **2008**, *60*, 1153.