Delivery of siRNA into B-Cell Lymphomas Using the Gene Pulser MXcell™ Electroporation System

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Introduction

The discovery of RNA interference (RNAi) has proven to be a powerful tool in post-transcriptional gene silencing by using small interfering RNAs (siRNAs). siRNAs are designed to target the mRNA with a complementary sequence in order to achieve degradation. Chemically synthesized 21-nucleotide siRNAs have the ability to induce RNA interference in mammalian cells.

Lymphomas are cancers derived from lymphocytes, a type of white blood cell that protects the organism from foreign bodies such as bacteria and viruses. B-cell lymphoma is primarily caused by chromosomal translocations and it constitutes approximately 85% of all non-Hodgkin lymphomas. RNAi is potentially useful in suppressing B-cell lymphomas. Currently, we are investigating possible genes that are over-expressed in B-cell lymphomas. Transfection of the siRNAs targeting these genes is crucial in order to determine if the knockdown is substantial, leading to treatment of B-cell lymphomas.

To test how synthetic siRNAs function, they must be transfected in the cell type of interest. Transfection of these synthetic siRNAs has been traditionally achieved by one of four methods: (1) cationic lipid–mediated transfection, (2) chemical-mediated transfection, (3) viral transfection, and (4) electroporation. Unfortunately B-cell lymphomas are extremely difficult to transfect by most traditional methods.

In this tech note, we describe the use of the Gene Pulser MXcell electroporation system to optimize transfection conditions for three different B-cell lymphoma cell lines: JEKO-1, SUDHL-6, and LY-1.

Methods

B-cell lymphoma cell line JEKO-1 and SUDHL-6 cells were maintained with RPMI medium 1640 (Irvine Scientific) and supplemented with 10% heat inactivated fetal bovine serum (FBS) and 2 mM of L-glutamine. LY-1 cells were maintained with IMDM medium (Irvine Scientific) and supplemented with 10% heat inactivated FBS and 2 mM of L-glutamine. JEKO-1, SUDHL-6, and LY-1 cells were suspended in Gene Pulser® electroporation buffer (Bio-Rad Laboratories, Inc.) at a density of 4 x 10^6–6 x 10^6 cells/ml, and the appropriate amounts were divided into two tubes, one for the control and one for the experiment. Each set consisted of buffer and cells, whereas the experimental tube also included 200 nM of hypoxanthine phosphoribosyltransferase 1 gene (*HPRT1*) synthetic siRNA. Each set was separately aliquoted in 500 µl fractions into the wells of a 24-well electroporation plate (Bio-Rad), then electroporated with the Gene Pulser MXcell system (Bio-Rad) using a resistance of 1,000 Ω and varying the waveform, voltage, capacitance, pulse duration, and number of pulses to determine the optimal condition for each cell type. The electroporated cells were transferred into a 6-well plate containing 2 ml of RPMI medium 1640 and incubated at 37°C with 5% CO_2.

Aliquots were taken from each set of transfected cells 48 hr post-transfection to determine cell viability microscopically, using trypan blue dye (NutriCyte Corporation). Samples with greater than 50% cell viability were processed further with RNA STAT-60 (Tel-Test, Inc.) to extract total RNA. The RNA samples were DNase I treated using a TURBO DNA-*free* kit (Ambion) and reverse transcribed into complementary DNA (cDNA) using random primers and Maloney murine leukemia virus reverse transcriptase (MMLV RT) (Invitrogen Corporation). An RNA sample of each preparation was processed without MMLV RT to provide a negative control in subsequent real-time PCR reactions. Quantitative analysis of *HPRT1* expression was determined by real-time PCR analysis with IQ™ SYBR® Green supermix (Bio-Rad), using a C1000™ thermal cycler (Bio-Rad). *HPRT1* expression was detected using 25 ng of cDNA, amplified with primers HPRT1-F (5'-TGACACTGGCAAAACAATGCA-3') and HPRT1-R (5'-GGTCCTTTTCACCAGCAAGCT-3'). Large ribosomal protein P0 (RPLP0) expression was detected using 25 ng of cDNA, amplified with primers RPLP0-F (5'-GGCGACCTGGAAGTCCAA-3') and RPLP0-R (5'-CCATCAGCACCACAGCCTTC-3'). The real-time PCR conditions were an initial denaturation at 95°C for 10 min, then 40 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 30 sec, and extension at 72°C for 30 sec, followed by a melt cycle. The level of *HPRT1* gene mRNA in each sample was normalized to the corresponding internal control RPLP0 content and recorded as a relative expression level.

Results

Various electroporation conditions were used to determine the best conditions for specific B-cell lymphoma cell line transfections (Table 1). Using the Gene Pulser MXcell electroporation system, we tested a total of 28 conditions by varying several parameters (waveform, voltage, capacitance, pulse duration, and number of pulses). Conditions that resulted in greater than 50% cell viability were used for RNAi transfection and gene knockdown analysis. Conditions that proved to have the highest *HPRT1* knockdown were repeated in three independent replicates to confirm both cell survival and gene knockdown results.

Table 1. Electroporation conditions.*

Condition	Waveform	Voltage, V	Duration, ms	Number of Pulses	Capacitance, Ω
1	Square	200	10	2	2,000
2	Square	200	25	1	2,000
3	Square	200	15	2	2,000
4	Square	200	20	1	2,000
5	Square	230	10	2	2,000
6	Square	230	20	1	2,000
7	Square	230	25	1	2,000
8	Square	250	10	1	2,000
9	Square	250	15	1	2,000
10	Square	250	15	2	2,000
11	Square	250	20	1	2,000
12	Square	300	20	1	2,000
13	Exponential	200	–**	–	250
14	Exponential	200	–	–	300
15	Exponential	200	–	–	325
16	Exponential	200	–	–	350
17	Exponential	225	–	–	250
18	Exponential	225	–	–	300
19	Exponential	225	–	–	350
20	Exponential	250	–	–	200
21	Exponential	250	–	–	250
22	Exponential	250	–	–	300
23	Exponential	250	–	–	350
24	Exponential	250	–	–	500
25	Exponential	250	–	–	750
26	Exponential	300	–	–	250
27	Exponential	300	–	–	300
28	Exponential	300	–	–	350

* Resistance was set to 1,000 Ω for all experiments.
** Indicates non-applicable parameters.

JEKO-1 cells showed high viability when subjected to conditions 1, 3, 5, and 13–16 (Figure 1A). It appears that conditions with two pulses with short durations (10 ms) permit the cells to recuperate from the electroporation better than when one pulse of longer duration (25 ms) is applied, as seen in the viability charts of JEKO-1 and LY-1. SUDHL-6 cells showed high viability with conditions 1, 2, 5, 6, 8–10, 13, 14, 16, and 21–23 (Figure 1B), while LY-1 cells showed high viability with conditions 1, 3–5, 13, 14, and 16 (Figure 1C). The conditions proven to elicit high viability were further analyzed to determine *HPRT1* knockdown. The conditions that produced the best *HPRT1* knockdown were 5, 13, 14, and 16 for JEKO-1 cells; 9 for SUDHL-6 cells; and 3 and 13 for LY-1 cells (Figure 2). Those conditions that demonstrated the best knockdown were repeated two additional times in order to derive a standard error for each of the repeated conditions (Figure 3). The standard errors proved to be minimal and thus the favorable conditions for each cell line can be trusted.

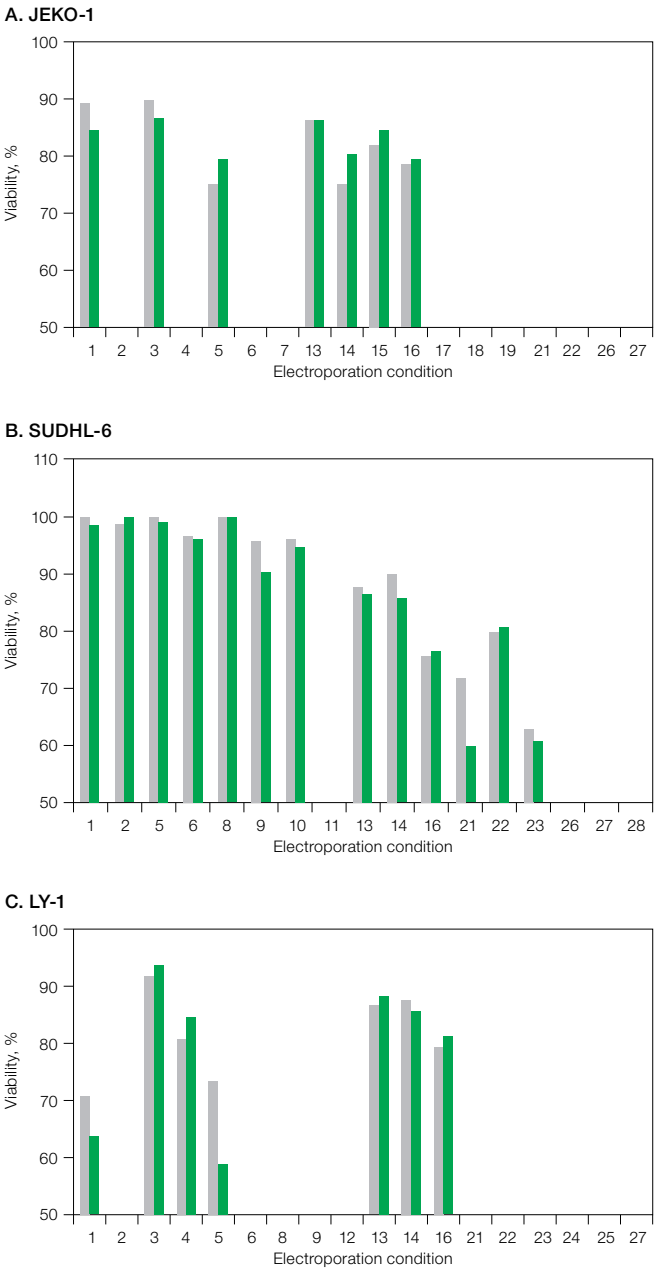

Fig. 1. Post-transfection viability. Post-transfection viability was measured using trypan blue dye. The electroporation conditions that were used are summarized in Table 1. ■, –siRNA; ■, +siRNA.

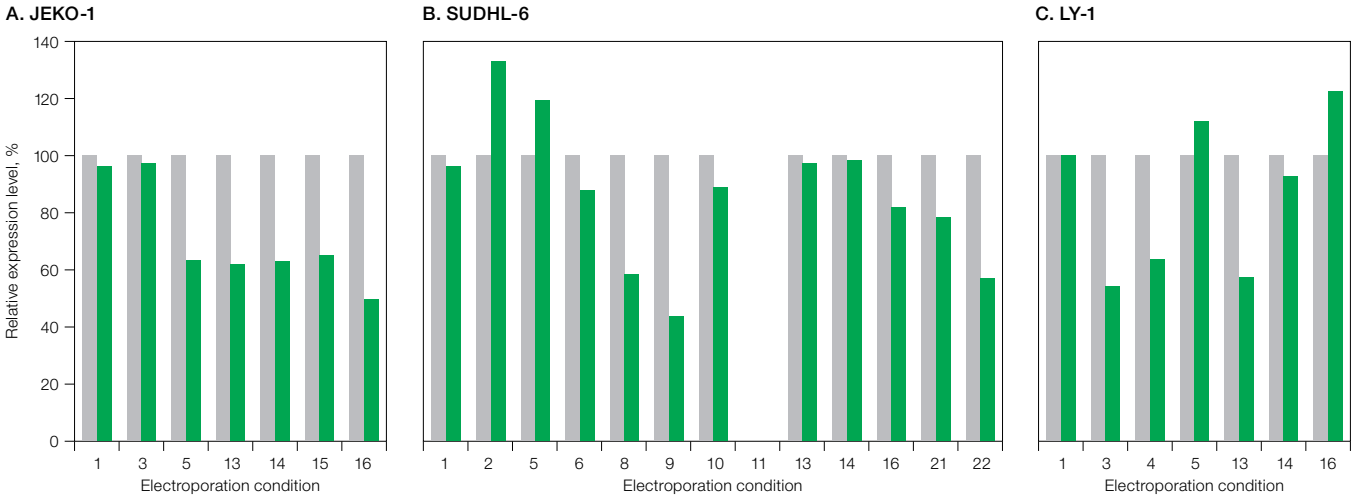

Fig. 2. Optimization for siRNA delivery. Knockdown of *HPRT1* was assessed by real-time PCR for each cell line. Electroporation conditions producing greater than 50% cell survival were used. ■, –siRNA; ■, +siRNA.

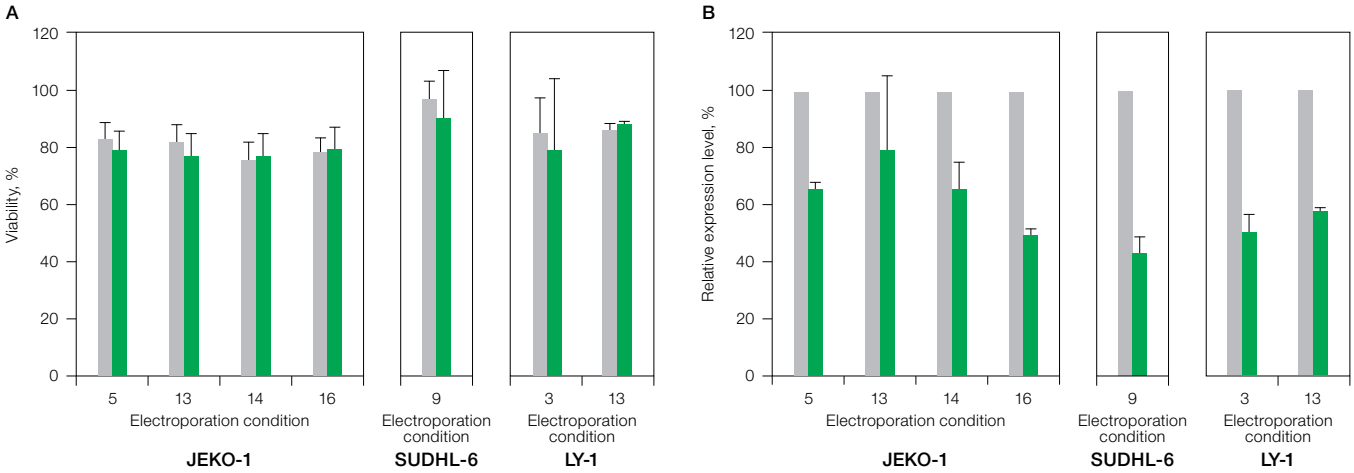

Fig. 3. Confirmation of optimal electroporation condition(s) for each cell line. A, post-electroporation survival rate; **B**, transfection efficiency. Each electroporation was performed three times. ■, –siRNA; ■, +siRNA.

Discussion

The Gene Pulser MXcell electroporation system was effective in transfecting B-cell lymphomas once the conditions resulting in minimal cell death were optimized. Initial electroporation conditions were selected from the preset protocols in the Gene Pulser MXcell system, and a range of conditions was then tested in order to determine the optimal condition for each cell type. Electroporation conditions 16, 9, and 3 produced the best siRNA delivery, with minimal cell death, in JEKO-1, SUDHL-6, and LY-1 cells, respectively.

The Gene Pulser MXcell electroporation system is a user-friendly system, and it can test multiple electroporation conditions in one plate.

Optimization of transfection conditions for B-cell lymphomas provides a gateway to combat this disease. siRNAs targeting over-expressed genes associated with B-cell lymphomas prove to be a promising therapeutic pathway since traditional treatments are inadequate.

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