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Research paper

Streptolysin-O reversible permeabilisation is an effective method to transfect siRNAs into myeloma cells

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Abstract

RNA interference (RNAi) has been shown to be a valuable tool to specifically target gene expression in a number of organisms becoming an indispensable weapon in the arsenal in functional genomics. In this study, we demonstrate that streptolysin-O (SLO) reversible permeabilisation is an efficient method to deliver small interfering RNAs (siRNAs) to hard-to-transfect human myeloma cell lines. We used published, pre-validated siRNAs for ERK2 and non-silencing siRNA control. We transfected siRNAs into human myeloma cell lines using SLO reversible permeabilisation method. Flow cytometry and western blot analysis were performed to assess the effect of SLO on transfection efficiency and ERK2 knockdown. These experiments demonstrate that SLO reversible permeabilisation method is an efficient and easy-to-use method to deliver siRNAs into human myeloma cell lines. Optimised SLO permeabilisation method showed to transfect >80% of JIM-3, H929, RPMI8226 and U266 cells, with minimal effect on cell viability (<10%) and cell cycle. Equally important, SLO permeabilisation induced a substantial knockdown of ERK2 at the protein level. These studies demonstrate that reversible SLO permeabilisation can successfully be applied to hard-to-transfect human myeloma cell lines to effectively silence genes.

Keywords: Multiple myeloma; Streptolysin-O; siRNA; Transfection

1. Introduction

Gene silencing by RNA interference (RNAi) has become a valuable tool in functional genomics, target validation and gene-specific therapeutic research. The use of RNAi in several organisms induces both strong and specific post-transcriptional gene silencing by directly mediating the degradation of RNA transcripts (Fire, 1999; Tuschl and Borkhardt, 2002; Sandy et al., 2005). RNA interference is triggered by short (<25 nucleotides) double stranded duplexes processed from longer RNA duplexes, called RNA precursors. RNA precursors give rise to two major classes of short RNAs, the microRNAs (miRNAs) and small interfering RNAs (siRNAs). Functionally, miRNAs form non-perfect Watson–Crick base pairing at the 3'UTR of target transcripts blocking translation. Whereas, siRNAs inhibit gene expressing by forming a perfect Watson–Crick base pairing along a target mRNA and inducing direct sequence-specific cleavage of the mRNA target (Sandy et al., 2005). These findings provided the base for the development of RNAi as a valuable tool in functional genomics, target validation and gene-specific therapeutic research.

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In the mammalian system the success of knocking down the expression of the gene of interest using siRNA duplexes mainly depends upon four factors: siRNA design, cell type, half-life of the target gene product and the delivery method (Sandy et al., 2005).

The use of RNAi technology in myeloma has been hampered by the inefficiency by which siRNA delivery methods promote the uptake of siRNA duplexes. However, the most successful siRNA delivery methods in myeloma are electroporation and viral gene transfer. Although, electroporation has been used with some success in some myeloma cell lines, the use of this technique has been shown to induce significant cell death (Croonquist and Van Ness, 2005; Zhu et al., 2005; Gomez-Benito et al., 2007). In addition, electroporation has only been shown to be of use in a limited number of myeloma cell lines (Croonquist and Van Ness, 2005; Zhu et al., 2005; Gomez-Benito et al., 2007). The use of viral gene transfer, on the other hand, has been shown to be a very effective way in inducing the knockdown of gene expression in myeloma cell lines (Hurt et al., 2004). However, viral gene transfer is time consuming as it requires cloning, virus production, transduction and marker selection, all of which have safety concerns and need a containment two laboratory facility. Thus, alternative methods to efficiently deliver siRNAs into MM cells with minimal effect on cell viability and do not require equipment are warranted.

Reversible SLO permeabilisation was shown to make several myeloid cell lines, such as KYO-1 and U937 amenable to antisense oligonucleotides, resulting in a significant reduction in expression of BCR-ABL, CMYC and MCL1 genes (Giles et al., 1995; Giles et al., 1998; Spiller et al., 1998; Moulding et al., 2000). Streptolysin-O (SLO), a bacterial cytolytic toxin is produced by several Gram-positive bacteria (Duncan and Schlegel, 1975). SLO molecules have been shown to bind to cholesterol in cytoplasmic membranes of animal cells (Palmer et al., 1998). Once bound to cholesterol, SLO monomers oligomerise into ring-shape structures giving rise to pores of ~30 nm of diameter (Duncan and Schlegel, 1975). The formation of SLO pores allows the flux of extracellular ions and macromolecules from the medium in which the cells are resuspended in, the SLO pore formation can be reversed by adding medium containing foetal calf serum (Palmer et al. embo 1998; Giles et al. 1998). Thus, allowing a controlled permeabilisation of plasma cell membranes. However, despite the use of the reversible SLO permeabilisation in suspension haematopoietic cell lines, its applicability as a mean to permeabilise hard-to-transfect myeloma cell lines to siRNA duplexes is unknown.

In this study we demonstrate that four myeloma cell lines JIM-3, H929, U266 and RPMI8226 are efficiently transfected with fluorescently labelled siRNA by SLO permeabilisation as measured by flow cytometry. In addition, SLO permeabilisation of myeloma cell lines showed to induce minimal cell death and high transfection efficiencies of 10–15% and 80–98%, respectively. Furthermore, SLO permeabilisation had a minimal effect on the cell cycle and induced a considerable knockdown of ERK2 expression in all myeloma cell lines tested. Our results clearly demonstrate that reversible SLO permeabilisation is the method of choice to efficiently deliver siRNA duplexes into myeloma cell lines.

2. Methods and Materials

2.1. Cell culture

JIM-3, U266, RPMI8226 and H929 myeloma cell lines were grown in the absence of antibiotics and mycoplasma contamination was excluded. All myeloma cell lines were cultured in RPMI-1640 medium (Invitrogen Life Technologies, Paisley, United Kingdom) supplemented with or without 10% foetal calf serum (Invitrogen Life Technologies, Paisley, United Kingdom). Cultures were maintained in exponential growth phase at 37 °C in a humidified atmosphere of 95% air/5% carbon dioxide.

2.2. siRNA duplexes

The siRNA duplex targeted against ERK2 and the non-silencing Alexa 488-labeled siRNA duplex were both obtained from Qiagen (Crawley, UK). The non-silencing Alexa 488-labeled siRNA duplex was targeted to AAT TCT CCG AAC GTG TCA CGT (sense: UUC UCC GAA CGU GUC ACG UdT dT; antisense: ACG UGA CAC GUU CGG AGA AdT dT). The targeted sequence of ERK2 was AAT GCT GAC TCC AAA GCT CTG (sense: UGC UGA CUC CAA UGC UCU GdT dT; antisense: CAG AGC UUU GGA GUC AGC AdT dT). Both duplexes were dissolved in siRNA suspension buffer (Qiagen, Crawley, UK) to a final concentration of 20 μM then incubated at 90 °C for 1 min followed by 60 min at 37 °C. Dissolved siRNA duplexes were aliquoted and stored at −20 °C.

2.3. Activation of Streptolysin O

Streptolysin O (Sigma Aldrich, Poole, UK) was dissolved in ddH_2O to 1000 Units/ml and activated with 5 mM ditiothreitol for 2 h at 37 °C, then checked for

activity and frozen in aliquots at −20 °C until required (Clark et al., 1999).

2.4. Transfection of myeloma cell lines

Myeloma cell lines growing exponentially were harvested washed twice with pre-warmed FCS-free RPMI-1640. The cells were resuspended in pre-warmed FCS-free RPMI-1640 at a concentration of 2×10^7/ml. 50 μl of cells in suspension were transferred to a flat-bottomed 96-well plate followed by the siRNA duplexes at a final concentration of 5 μM. Cells were made permeable to siRNA duplexes by adding varying concentrations of streptolysin-O (JIM-3: 6U, H929:8U, U266: 8U and RPMI8226: 10U) and immediately mixed followed by 10 min incubation at 37 °C, during which cells were mixed twice. 250 μl of pre-warmed RPMI-1640 supplemented with 10% FCS was added to stop the transfection followed by a 30 min incubation at 37 °C. Cells were transferred to a 6-well plate containing pre-warmed RPMI-1640 supplemented with 10% FCS. ERK2 protein knockdown was assessed 48 h post-permeabilisation by western blotting analysis.

2.5. Transfection efficiency

Twenty four hour post-transfection, cells were washed twice in PBS and finally resuspended in PBS containing 1%(v/v) of foetal calf serum. Transfection efficiency was analysed by flow cytomery using Alexa 488 fluorescently labelled negative control siRNA duplex. Cell cultures were analysed using a LSR II flow cytometer equipped with FACSDiva software (BD Biosciences).

2.6. Cell cycle and apoptotic analysis

Cell cycle analysis of cells permeabilised with the negative control siRNA duplex was performed at 24 h post-transfection using propidium iodide staining and flow cytometry (BD™ LSR II) with FACSDiva software (BD Biosciences).

For studies of apoptosis, cells were analysed 24 h after siRNA transfection. Apoptosis was determined by Annexin-V staining (Bender MedSystems GmbH, Vienna, Austria) and analysed by flow cytometry.

2.7. Cell viability

The effect of varying concentrations of streptolysin-O on cell viability was estimated 24 h post-transfection by trypan blue dye exclusion using a haemocytometer.

2.8. Western blotting analysis

Whole cell lysates were made by washing the MM cell lines in cold PBS followed by lysis in cell lysis buffer (50 mM Tris–HCl, pH 7.5; 150 mM NaCl; 1% (v/v) Triton X-100 +0.5% (w/v) Na-Deoxycholate; 1 mM EDTA; 1 mM PMSF) containing a cocktail of protease inhibitors (Roche Applied Science, UK). Protein concentration was estimated by using the BCA™ Protein Assay Kit (Pierce Biotechnology, Inc., Rockford, US). Twenty micrograms of total proteins were fractionated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with primary (Taq-100, Qiagen, Crawley, UK) and secondary (GE Healthcare, UK) antibodies followed by chemiluminescence detection using ECL Plus™ (GE Healthcare, UK). Blots were stripped and immunoblotted with anti-GAPDH antibody (FL-335, Santa Cruz Biotechnology, UK) to control for protein loading.

3. Results

3.1. Myeloma cell lines are efficiently transfected with siRNA

The use of RNAi technology to elucidate the biological role of genes in the pathogenesis of myeloma has been hindered due to the reluctance of myeloma cells to uptake siRNAs by chemical transfection or electroporation. This in turn, has led to the use of the time consuming and safety adverse–viral gene transfer system — to deliver siRNAs into myeloma cells. In order to overcome the disadvantages of viral transfer system and develop a comparatively cheap and easy method to make myeloma cells amenable to siRNAs, we evaluated the use of streptolysin-O as a means to reversibly permeabilise myeloma cells to siRNA duplexes. To determine the permeabilisation efficiency and uptake of siRNA duplexes, a non-silencing fluorescently labelled siRNA duplex was used. Four myeloma cells lines were washed in FCS-free RPMI-1640 medium and made permeable to 5 μM of fluorescently labelled siRNA duplex by different concentrations of SLO. Immediately after SLO permeabilisation, cells were transferred to RPMI-1640 medium supplemented with 10% FCS. Transfection efficiency was determined 24 h post-transfection by flow cytometry analysis.

As shown in Figs. 1 and 2 the siRNA was efficiently delivered to all myeloma cell lines used. The uptake efficiency of siRNA by U266, RPMI8226, JIM-3 and H929 myeloma cell lines was 81%, 98%, 97% and 95%, respectively, when compared to cells incubated with the siRNA in the absence of SLO. These data clearly

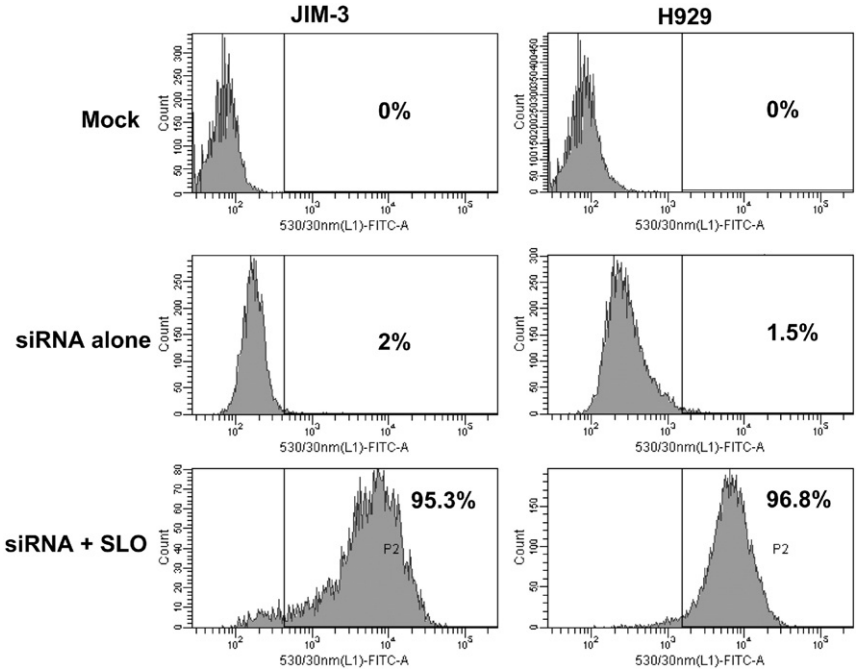

Fig. 1. A non-silencing Alexa488-labelled siRNA duplex was efficiently transfected into myeloma cells lines. Myeloma cell lines, JIM-3 and H929, were made permeable to the fluorescently labelled siRNA by incubating with 6U and 8U of SLO. SLO permeabilisation led to a transfection efficiency of >85% cells. The graphs show a typical result of three independent experiments from an ungated population.

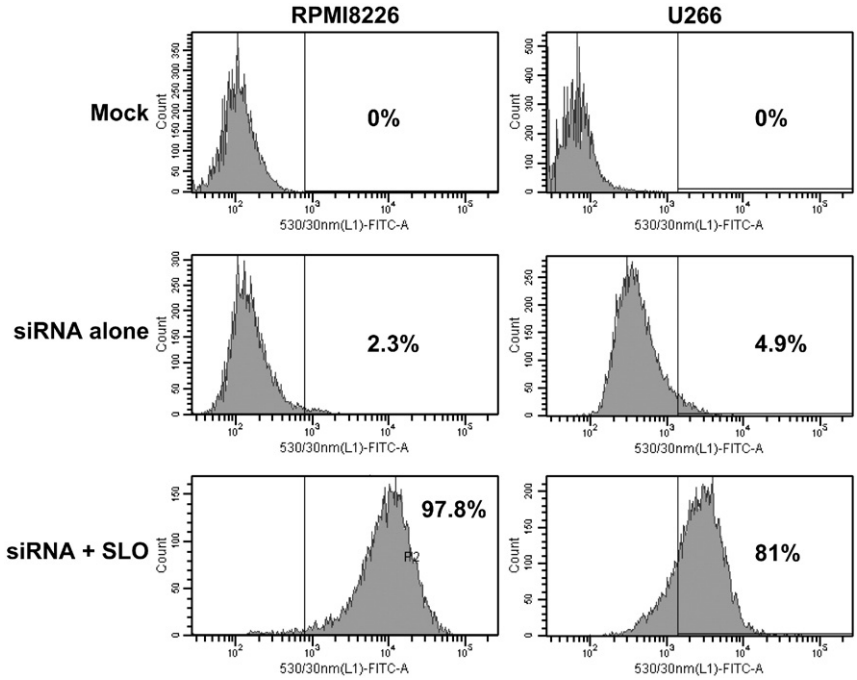

Fig. 2. A non-silencing Alexa488-labelled siRNA duplex was efficiently transfected into myeloma cells lines. Myeloma cell lines, U266 and RPMI8226 were made permeable to the fluorescently labelled siRNA by incubating with 8U and 10U of SLO. SLO permeabilisation led to a transfection efficiency of >85% cells. The graphs show a typical result of three independent experiments from an ungated population.

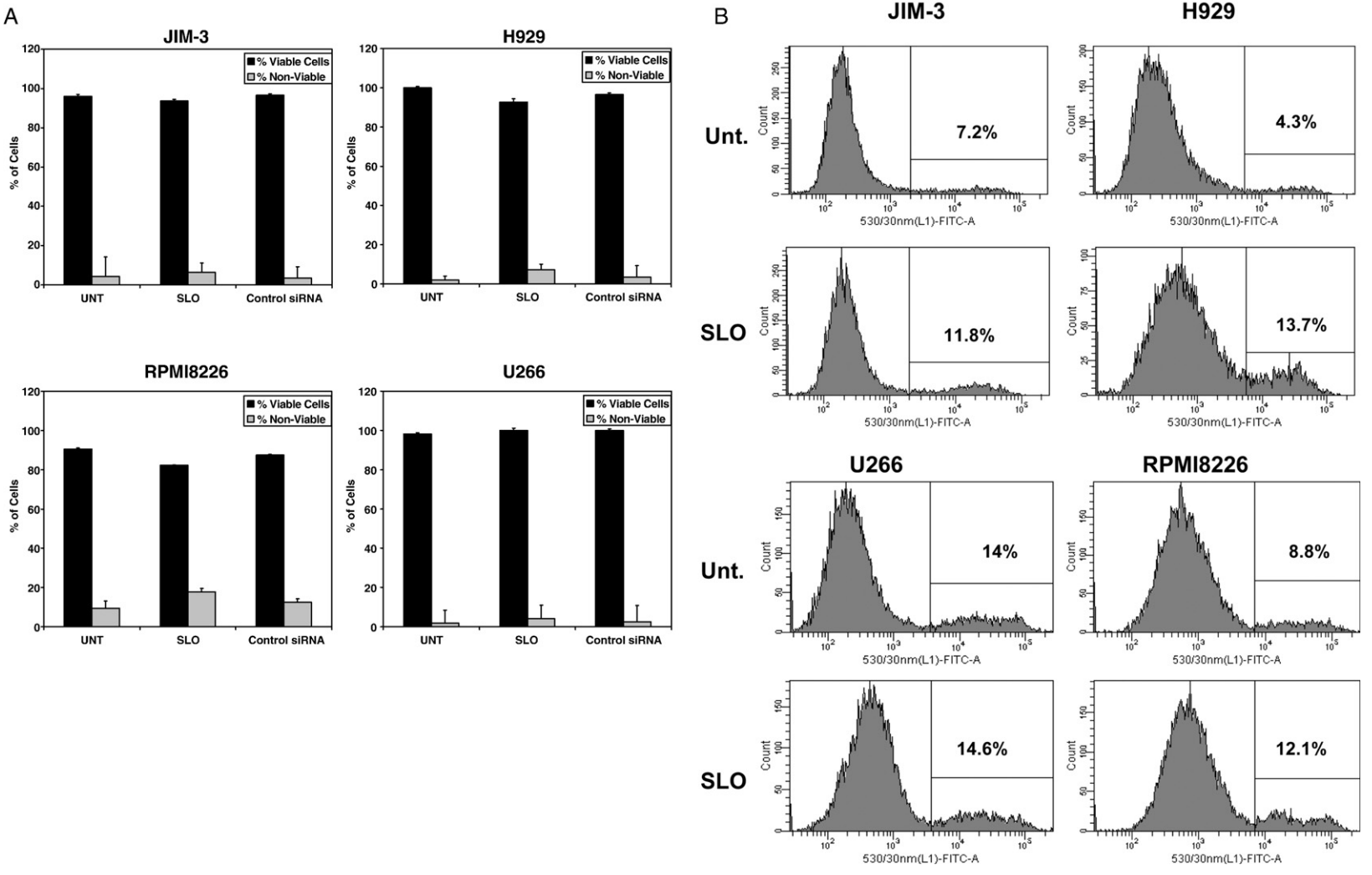

Fig. 3. SLO permeabilisation of myeloma cell lines does not lead to a strong increase in the number of dead cells after 24 h. (A) Myeloma cell lines, JIM-3, H929, U266 and RPMI8226 were incubated with or without SLO, in addition, SLO exposed cells were incubated with or without the non-silencing Alexa488-labelled siRNA duplex and cell viability was assessed by trypan blue dye exclusion. The columns represent the percentage of cells that were either viable or non-viable. The average (±SD) of three independent experiments is shown from each myeloma cell line. (B) The affect of SLO permeabilisation with or without the presence of non-silencing siRNA duplex was also assessed by Annexin V staining. The data is a representative of three independent experiments for ungated JIM-3, H929, U266 and RPMI8226 cell lines.

demonstrate that SLO treatment permeabilises myeloma cell lines to siRNAs.

3.2. Transfection of myeloma cells with siRNA does not compromise viability

One of the prerequisites of a successful siRNA delivery system is to induce a high uptake of siRNA duplexes without being detrimental to the cell viability. In order to assess the impact of the SLO permeabilisation on the viability of myeloma cell lines, cells were exposed to SLO with or without 5 μM of fluorescently labelled non-targeting siRNA duplex. Cell viability was then determined 24 h after permeabilisation by trypan blue dye exclusion using a haemocytometer. As shown in Fig. 3A, SLO permeabilisation did not induce a significant increase in the number of trypan blue positive cells in JIM-3, H929, U266 and RPMI8226 cell lines (6.4%±4.7; 7.4%±2.7%; 17.7%±1.7% and 4%±4.7%) when compared to the untreated control (4.1%±9.9%; 2.1%±2%; 9.5%±3.6% and 1.7%±6.8%). In addition, the viability of JIM-3, H929, U266 and RPMI8226 cell lines was not affected by the SLO permeabilisation whether the non-silencing fluorescently labelled siRNA duplex was present when compared to the untreated control.

To further determine the effect of SLO permeabilisation on myeloma cell lines Annexin-V staining was performed on JIM-3, H929 and RPMI8226 lines. As shown in Fig. 3B, SLO permeabilisation did not lead to an increase in the number of cells undergoing apoptosis when compared to the control. These data clearly demonstrate that permeabilisation of myeloma cells to siRNAs by SLO does not compromise cell viability. In addition, it further confirms the data obtained by trypan blue dye exclusion.

3.3. Permeabilisation of myeloma cells by SLO does not affect cell cycle

To ensure that SLO permeabilisation does not affect the cell cycle of myeloma cells, we performed cell cycle analysis by flow cytometry on JIM-3, H929 and RPMI8226 myeloma cells 24 h after being exposed to

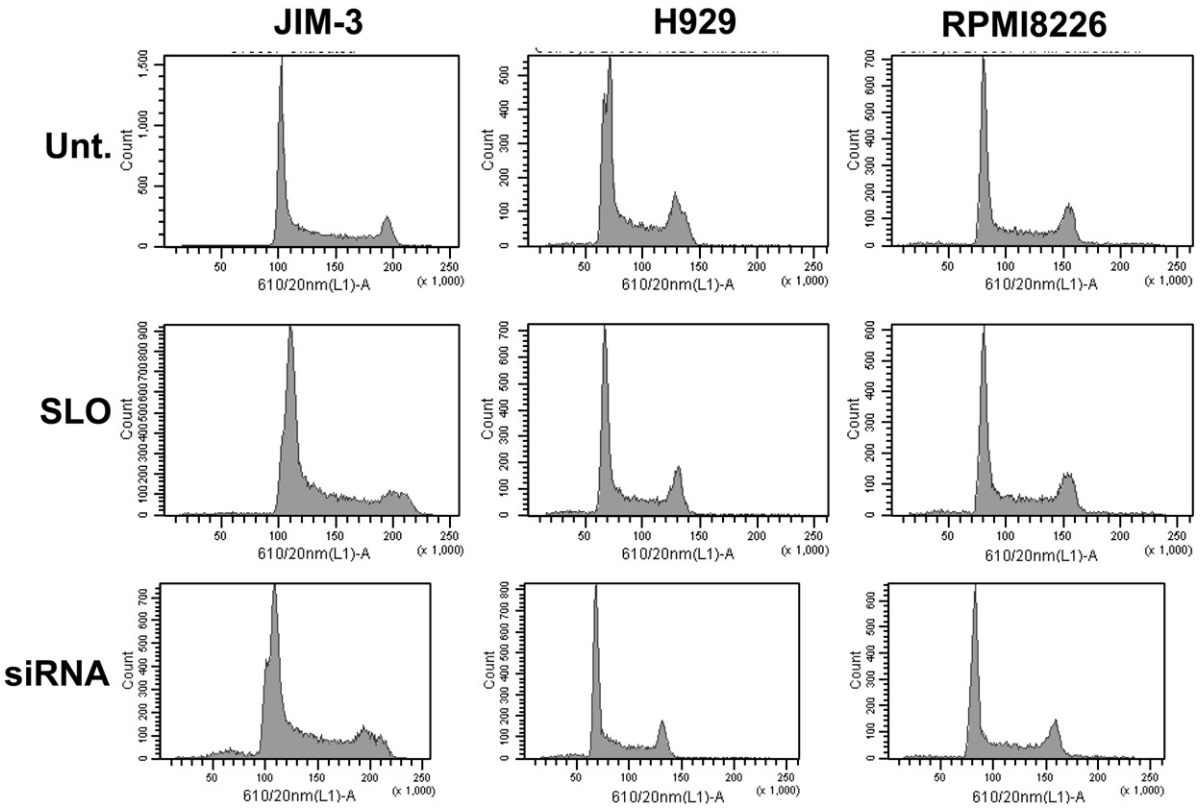

Fig. 4. SLO permeabilisation of myeloma cell lines does not affect cell cycle after 24 h. Myeloma cell lines, JIM-3, H929 and RPMI8226 were incubated with or without SLO, in addition, SLO exposed cells were incubated with or without the non-silencing Alexa488-labelled siRNA duplex and cell cycle analysis was performed by propidium iodide staining 24 h post-permeabilisation. The data is a representative of three independent experiments from an ungated population.

SLO. As shown in Fig. 4, permeabilisation of myeloma cells by SLO did not induce significant changes of the cell cycle in all cell lines tested when compared to the untreated controls. In addition, the uptake of negative control siRNA duplex had no effect on the cell cycle. These data clearly demonstrate that the use of SLO to make myeloma cell amenable to exogenous molecules does not affect the cell cycle. Furthermore, it demonstrates that the siRNA duplex used is a useful tool to assess the transfection efficiency and to be used as a negative control.

3.4. siRNA uptake induces a knockdown of ERK2 expression in myeloma cells

In order to demonstrate that siRNA duplexes are intact when delivered to myeloma cell lines by SLO permeabilisation and knockdown gene expression, we used a pre-validated siRNA duplex known to target the expression of ERK2 from the RAS-MAPK signalling pathway (Haughton et al., 2006). Furthermore, the siRNA duplex against ERK2 was chosen because as it is commercially available as positive controls for siRNA experiments (Qiagen, Crawley, UK).

All four myeloma cell lines were permeabilised to 5 μM of ERK2 siRNA using varying concentrations of SLO. After 48 h the total protein lysates were isolated, and the knockdown was verified at the protein level by western blotting analysis. To exclude unequal loading of protein lysates the level of GAPDH protein was also monitored by western blotting analysis.

As shown in Fig. 5, uptake of the ERK2 siRNA duplex induced an efficient knockdown of ERK2 protein level in JIM-3, H929, U266 and RPMI8226 myeloma cell lines. In addition, it was observed that SLO permeabilisation with or without siRNA duplexes did not affect the expression of GAPDH. These data demonstrate that SLO permeabilisation is an effective delivery method to transiently silence gene expression in myeloma cells.

4. Discussion

The use of myeloma cell lines is of uttermost importance in a preclinical setting, especially in target validation and in functional genomics. The use of the RNAi methodology in myeloma cells has been hindered by the lack of cost effective and technically undemanding siRNA delivery methods, with most either delivering insufficient siRNAs or inducing a significant cell death. The current siRNA delivery methods that have delivered siRNA duplexes into myeloma cell lines with an effect on the expression of the target gene are primarily electroporation and viral transduction (Hurt et al., 2004; Croonquist and Van Ness, 2005; Zhu et al., 2005; Gomez-Benito et al., 2007). The former method has been shown to not only be of limited use to specific myeloma cell lines, but also induce significant cell death (Zhu et al. 2005; Gomez-Benito et al., 2007). In addition, electroporation requires the purchase and use of an electroporator, which may not be cost effective if a limited number of electroporations are planned. More recently, companies are developing optimised electroporation buffers for specific cells and that can solely be used with their manufactured electroporators. This is very useful if the cell lines that are to be used in a study

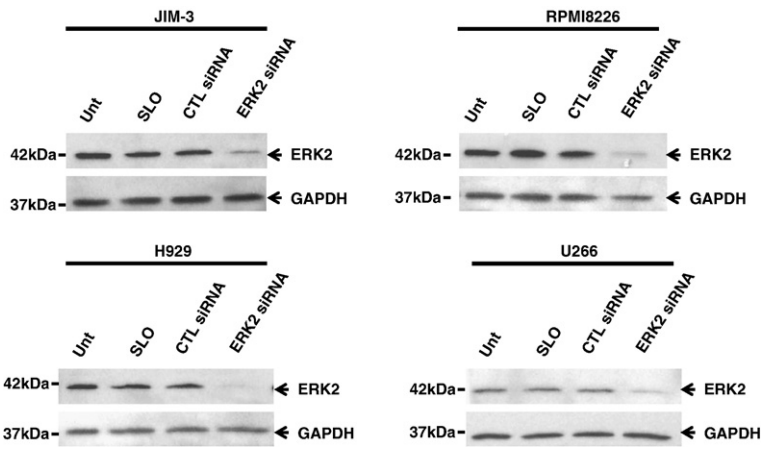

Fig. 5. ERK2 siRNA duplex knockdowns ERK2 expression in JIM-3, H929, U266 and RPMI8226 myeloma cell lines. Myeloma cell lines were incubated to 5 μM of siRNA duplex in the presence of varying concentrations of SLO as detailed in Materials and Methods. The ERK2 protein knockdown was monitored by Western blotting analysis. The level of GAPDH protein was used to monitor for equal loading.

have been optimised by such company. Conversely, if the cells to be used have not been previously optimised, then other delivery methods have to be considered. The use of viral transduction, on the other hand, has been shown by several studies to be a very effective way to knockdown gene expression in myeloma cells (Hurt et al., 2004; Guo et al., 2006; Gomez-Benito et al., 2007). However, the use of viral transduction in knockdown studies involves dealing with time consuming cloning, viral packaging, transduction, marker selection and the need of containment 2 laboratory, in addition, safety concerns exist.

One of the main aims of this study was to determine the applicability and feasibility of streptolysin-O (SLO) as a mean to permeabilise hard-to-transfect suspension myeloma cell lines to exogenous siRNA duplexes. In the present study we were able to demonstrate that SLO premeabilisation is an effective and straightforward delivery method to knockdown the expression of genes in myeloma cell lines.

The first step of a successful siRNA delivery method is to make cells amenable to exogenous siRNA duplexes. With this point in mind, we investigated whether SLO treatment could promote the uptake of a fluorescently labelled non-silencing siRNA duplex into a panel of myeloma cell lines. SLO treatment was found to deliver the siRNA duplex (>80% transfection efficiency) to four human myeloma cell lines (Figs. 1 and 2). Electroporation and SLO permeabilisation both induce pore formation by different mechanisms allowing the influx of exogenous siRNA duplexes into the cells. Despite the fact that electroporation was shown some success as a siRNA delivery method in some myeloma cell lines, it induces substantial cell death (Zhu et al., 2005; Gomez-Benito et al., 2007). However, unlike electroporation, SLO permeabilisation with or without the non-silencing siRNA duplex minimally affected cell viability and cell cycle (Figs. 3 and 4).

Finally, we evaluated the efficiency of ERK2 knockdown in the panel of myeloma cell lines using SLO permeabilisation. ERK2 was chosen as a target as the siRNA duplex of this transcript has been previously reported to efficiently silence ERK2 expression (Haughton et al., 2006). As expected, SLO permeabilisation of myeloma cells to the siRNA duplex induced an efficient and specific knockdown of ERK2 expression (Fig. 5) and was in agreement with the SLO permeabilisation efficiency as determined by the fluorescently labelled non-silencing siRNA (Figs. 1 and 2). In summary, we describe here that reversible SLO permeabilisation is suitable for efficient delivery of siRNA duplexes into hard-to-transfect myeloma cells. In addition, we describe that reversible SLO permeabilisation is an easy-to-use technique that does not require specialised equipment to effectively silence gene expression.

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References

Clark, R.E., Grzybowski, J., Broughton, C.M., Pender, N.T., Spiller, D.G., Brammer, C.G., Giles, R.V., Tidd, D.M., 1999. Clinical use of streptolysin-O to facilitate antisense oligodeoxyribonucleotide delivery for purging autografts in chronic myeloid leukaemia. Bone Marrow Transplant. 23, 1303.

Croonquist, P.A., Van Ness, B., 2005. The polycomb group protein enhancer of zeste homolog 2 (EZH 2) is an oncogene that influences myeloma cell growth and the mutant ras phenotype. Oncogene 24, 6269.

Duncan, J.L., Schlegel, R., 1975. Effect of streptolysin O on erythrocyte membranes, liposomes, and lipid dispersions. A protein-cholesterol interaction. J. Cell. Biol. 67, 160–174.

Fire, A., 1999. RNA-triggered gene silencing. Trends Genet. 15, 358–363.

Giles, R.V., Ruddell, C.J., Spiller, D.G., Green, J.A., Tidd, D.M., 1995. Single base discrimination for ribonuclease H-dependent antisense effects within intact human leukaemia cells. Nucleic Acids Res. 23, 954.

Giles, R.V., Spiller, D.G., Grzybowski, J., Clark, R.E., Nicklin, P., Tidd, D.M., 1998. Selecting optimal oligonucleotide composition for maximal antisense effect following streptolysin O-mediated delivery into human leukaemia cells. Nucleic Acids Res. 26, 1567.

Gomez-Benito, M., Balsas, P., Carvajal-Vergara, X., Pandiella, A., Anel, A., Marzo, I., Naval, J., 2007. Mechanism of apoptosis induced by IFN-alpha in human myeloma cells: role of Jak1 and Bim and potentiation by rapamycin. Cell. Signal. 19, 844.

Guo, C., Hou, J., Chen, Y.B., Jin, H.Y., Fu, W.J., Yuan, Z.G., Wang, D.X., 2006. The effect of cyclin D2 shRNA on the proliferation and apoptosis of LP-1 cell. Zhonghua Xue Ye Xue Za Zhi 27, 666.

Haughton, E.L., Tucker, S.J., Marek, C.J., Durward, E., Leel, V., Bascal, Z., Monaghan, T., Koruth, M., Collie-Duguid, E., Mann, D.A., Trim, J.E., Wright, M.C., 2006. Pregnane X receptor activators inhibit human hepatic stellate cell transdifferentiation in vitro. Gastroenterology 131, 194.

Hurt, E.M., Wiestner, A., Rosenwald, A., Shaffer, A.L., Campo, E., Grogan, T., Bergsagel, P.L., Kuehl, W.M., Staudt, L.M., 2004. Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. Cancer Cell. 5, 191.

Moulding, D.A., Giles, R.V., Spiller, D.G., White, M.R., Tidd, D.M., Edwards, S.W., 2000. Apoptosis is rapidly triggered by antisense depletion of MCL-1 in differentiating U937 cells. Blood 96, 1756.

Palmer, M., Harris, R., Freytag, C., Kehoe, M., Tranum-Jensen, J., Bhakdi, S., 1998. Assembly mechanism of the oligomeric

streptolysin O pore: the early membrane lesion is lined by a free edge of the lipid membrane and is extended gradually during oligomerization. Embo J. 17, 1598.

Sandy, P., Ventura, A., Jacks, T., 2005. Mammalian RNAi: a practical guide. Biotechniques 39, 215.

Spiller, D.G., Giles, R.V., Grzybowski, J., Tidd, D.M., Clark, R.E., 1998. Improving the intracellular delivery and molecular efficacy of antisense oligonucleotides in chronic myeloid leukemia cells: a comparison of streptolysin-O permeabilization, electroporation, and lipophilic conjugation. Blood 91, 4738.

Tuschl, T., Borkhardt, A., 2002. Small interfering RNAs: a revolutionary tool for the analysis of gene function and gene therapy. Mol. Interv. 2, 158.

Zhu, L., Somlo, G., Zhou, B., Shao, J., Bedell, V., Slovak, M.L., Liu, X., Luo, J., Yen, Y., 2005. Fibroblast growth factor receptor 3 inhibition by short hairpin RNAs leads to apoptosis in multiple myeloma. Mol. Cancer Ther. 4, 787.