siRNA transfection of A549 cells with Metafectene SI and downregulation of Hedgehog Acyltransferase (Hhat) using an siRNA pool.

Antonio Konitsiotis,
Imperial College London,
Section of Molecular Medicine/NHLI,
Sir Alexander Fleming Building,
Floor 2, Desk 7,
London SW7 2AZ,
UK.
e-mail: antonios.konitsiotis05@imperial.ac.uk

Introduction

The aim of this study was to compare the efficiency of internalisation of fluorescent siRNA molecules following transfection of A549 lung cancer cells with three different liposomal transfection reagents, Metafectene SI, FuGENE HD and Lipofectamine RNAiMAX. The optimal transfection reagent was determined to be Metafectene SI, which was then used in siRNA knockdown experiments of Hedgehog Acyltransferase (Hhat) in A549 cells. In conclusion, Metafectene SI provided the highest siRNA transfection efficiency in these cells while also providing efficient knockdown of Hhat expression.

Materials and Methods

Materials

siGLO Laminin A/C control siRNA and the human MART 2 siRNA on TARGET SMART pool were purchased from Dharmacon (UK). Transfection reagents used were Metafectene SI (Biontex, Germany), FuGENE HD (Promega, UK) and Lipofectamine RNAiMAX (Invitrogen, UK). GoTaq® 2-Step RT-qPCR System was purchased from Promega (UK).

Cells

The human alveolar adenocarcinoma cell line A549 (gift from Simak Ali, Imperial College London) was cultured in RPMI 1640 (PAA, UK) supplemented with 10% FCS (PAA, UK) at 37^{o}C, 5% CO_2.

Transfection protocols

Reverse transfection

Experiments were performed either in 12-well or 6-well cell culture plates (Nunc, Denmark). In each well the transfection reagents were prepared as to the manufacturer's instructions. In the meantime, subconfluent A549 cells were harvested and 1.5 x 10^5 (12-well) or 2.5 x 10^5 (6-well) cells were seeded to each well. Cells were then incubated at 37^{o}C for up to 72hrs.

Forward transfection

Experiments were performed either in 12-well or 6-well cell culture plates (Nunc, Denmark). Subconfluent A549 cells were harvested and 1x 10^5 (12-well) or 2 x 10^5 (6-well) cells were seeded

into each well and incubated at 37^{o}C. The next day cells were ~70% confluent. Transfection reagents were prepared in sterile 1.5ml tubes (Eppendorf) as to the manufacturer's instructions and then added to the wells in a dropwise manner. Cells were then incubated at 37^{o}C for up to 72 hrs post-transfection.

Results and discussion

Transfection efficiency of A549 cells with the fluorescent siRNA molecule siGLO was determined by fluorescence microscopy at a magnification of 25x using a blue fluorescent filter (data not shown). From the three liposomal transfection reagents tested, Metafectene SI, FuGENE HD and Lipofectamine RNAiMAX, the highest transfection efficiency was achieved with Metafectene SI, with efficiency being approximately 70%. Second best transfection efficiency was achieved with Lipofectamine RNAiMAX (~40-50%) while FuGENE HD gave poor transfection results with siGLO (data not shown). With regards to cytotoxicity, Metafectene SI had the highest cytotoxic effect, with many cells dying after 72hrs if the medium was not changed. However, this was alleviated by exchanging with fresh medium 12-16hrs post-transfection, with no effect on transfection efficiency. Finally, both forward and reverse transfection protocols gave similar results, although signal seemed highest when reverse transfection protocol was performed.

Experiments were conducted with Metafectene SI to test the downregulation of Hedgehog acyltransferase (Hhat) in the A549 cells, as determined by qPCR. Cells in 12-well culture plates were reverse transfected with the MART 2 siRNA pool (Dharmacon), which knocks down human Hhat, or a non-targeting siRNA pool (Dharmacon). RNA was then extracted from the cells using TriZOL reagent (Invitrogen, UK, according to the manufacturer's instructions. Following RNA extraction, RT-PCR and qPCR, using Hhat-specific primers, was performed using the GoTaq® 2-Step RT-qPCR System (Promega) in 96-well qPCR plates (Applied Biosystems) using the Applied Biosystems 7500 Real-Time PCR system. Results were analysed by the ΔΔCt method (Livak method) and normalised against either the GAPDH (Figure 1a) or RPLPO (Figure 1b) housekeeping genes.

Results revealed that, compared to the non-targeting scrambled siRNA pool, efficient downregulation of up to 74% of Hhat expression was achieved in A549 cells transfected with a Dharmacon Hhat siRNA pool using Metafectene SI. This result is comparable to previous results achieved with other transfection reagents (data not shown).

Conclusion

Metafectene SI is a powerful siRNA transfection reagent that provided higher transfection efficiency levels with a fluorescent siRNA molecule compared to two other popular transfection reagents. Cell toxicity was an issue; however, it was manageable and less pronounced if the medium was exchanged with fresh medium. Finally, there was no difference in transfection efficiency between reverse and forward transfection methodologies, and efficient Hhat downregulation was achieved in A549 cells transfected with a Hhat siRNA pool using Metafectene SI.

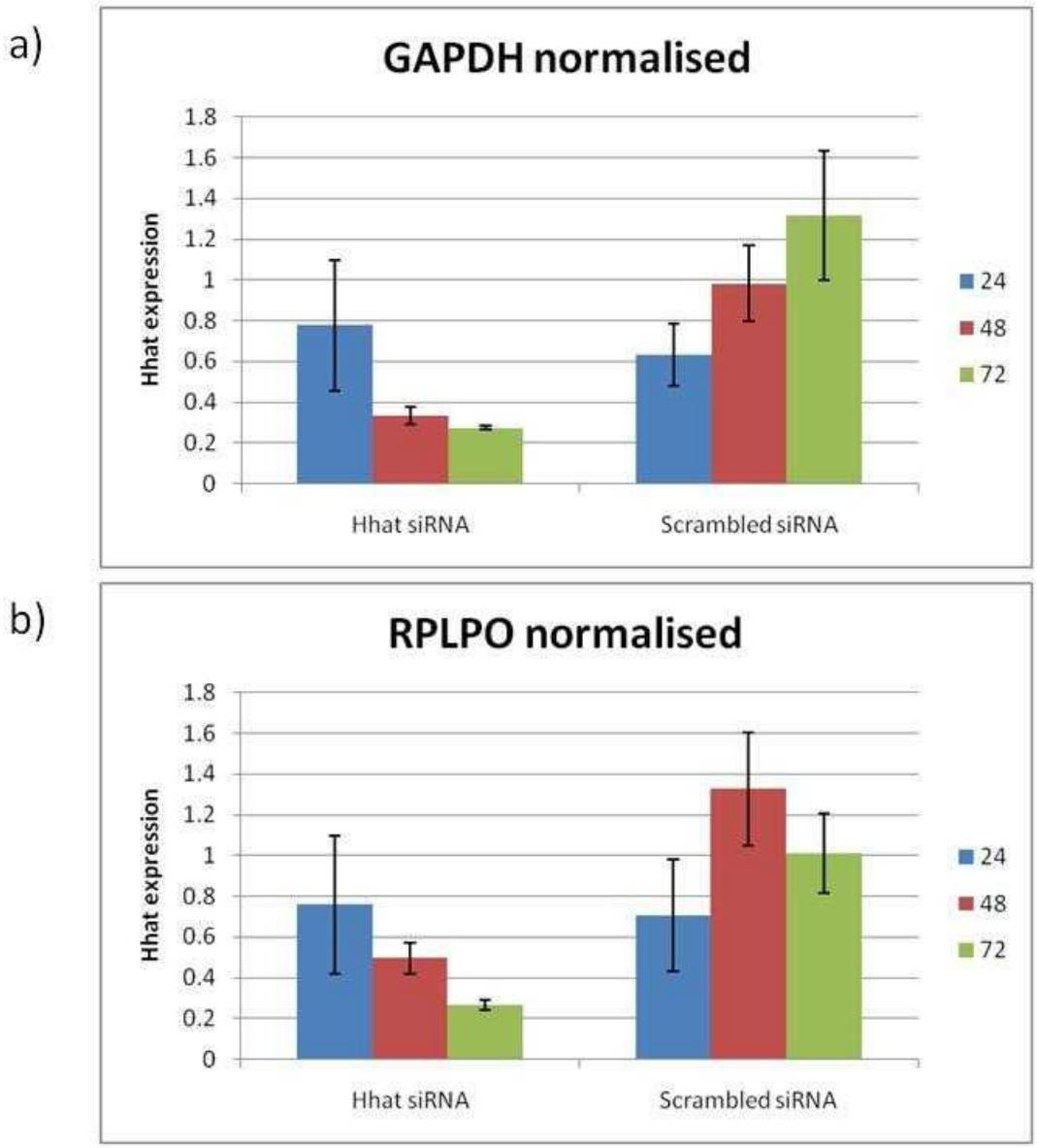

Figure 1: Hhat expression results from 3 separate experiments performed in duplicate, +/- SE. A549 cells were transfected with either an Hhat targeting siRNA pool (Hhat siRNA) or a non-targeting siRNA pool (Scrambled siRNA). RNA was extracted at 24, 48 and 72 hrs post-transfection and RT-PCR and qPCR performed on these samples. During the qPCR experiments samples were probed for two different reference genes, GAPDH (**a**) and RPLPO (**b**), which were used to normalise the results. In both cases Hhat is significantly downregulated at 48 and 72 hrs post-transfection ($p<0.05$). At 72hrs Hhat expression was at 26% compared to the scambled siRNA transfected control.