Application Note:

Title of Experiment:

Silencing expression of the transcriptional repressor GFI1 in primary human CD8+ T cells.

Author, Institute and address:

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Introduction:

(Leads: What was the incitement of your experiment? What cell lines or domains in transfection did you investigate and why?)

Previous results from our lab indicated an increase in the expression of GFI1 in a subset of CD8+ T cells isolated from human peripheral blood mononuclear cells (PBMCs). Therefore we wanted to determine the role of GFI1 by silencing its expression in primary CD8+ T cells. We chose to test the ability of Metafectene Pro to silence GFI1 expression by transfecting with GFI1 target specific siRNA.

Materials and methods:

BD Vacutainer Sodium Heparin tubes / Vacutainer Blood collection set (21G ¾ x 12")

PBMCs

Ficoll-Paque PLUS and instructions

1xPBS

AutoMACS / Buffers indicated in instruction manual

Miltenyi Biotec: human CD8+ T cell isolation kit II and protocol

Complete Iscove modified Dulbecco's medium (cIMDM final [10% FCS, 0.2% pen/gen]; supplemented with FCS and penicillin/gentamycin)

24 well-plate

Beckman Coulter Allegra X-12R centrifuge

Metafectene Pro

Non-silencing (NS) and study siRNA targeting GFI1 designated G1 and G4 (Qiagen)

Pipettor /glass pipets / pipet tips etc…

Incubator (37^0C / 5% CO_2)

Fridge 4^oC

7500 Real Time PCR System (Applied Biosystems); primers for real time and TaqMan Master mix (Applied Biosystems)

BD FACSCanto (BD Biosciences); antibodies for flow cytometry: BD Biosciences and Santa Cruz

RNEasy extraction kit (Qiagen)

Reverse Transcription kit (Applied Biosystems)

Experimental procedures / transfection protocol:

1. Isolation of PBMCs as indicated in Ficoll-Paque information / protocol sheet.

2. Isolation of CD8+ T cells as indicated in human CD8+ T cell isolation kit II protocol.

3. Transfection was conducted as described in the Metafectene Pro instruction manual.

    a. Transfection of siRNA – Protocol for initial optimization in a 24 well-plate

        i. Cell number used was 1.6 x 10^5.

        ii. Condition for 0.5 µg of siRNA were evaluated.

    b. Upscaling is being conducted as indicated in section 4 for 1x10^6 and 2x10^6 cells (data has not yet been available).

    Transfection mixture was removed after 5 hours by centrifuging at 140 x g for 8 minutes at room temperature.

4. Cells were collected at approximately 24 hours for flow cytometry or for quantitative Real Time PCR.

Results and discussion:

(Leads: comparison results from METAFECTENE or other reagents? Enhance of previous transfection efficiency with METAFECTENE PRO?)

Low cell death

Efficient transfection

Conclusion / summary:

Metafectene Pro efficiently reduced GFI1 mRNA expression by approximately 80% while maintaining high cell viability at 24 hours. Furthermore, GFI1 protein expression was significantly reduced as visualized by flow cytometry.

References:

N/A

Appendix: Tables and/or figures:

Figure 1. GFI1 gene expression following its silencing in human primary CD8+ T cells using siRNA G1 and G4 approximately 24 hours following transfection (n=3).

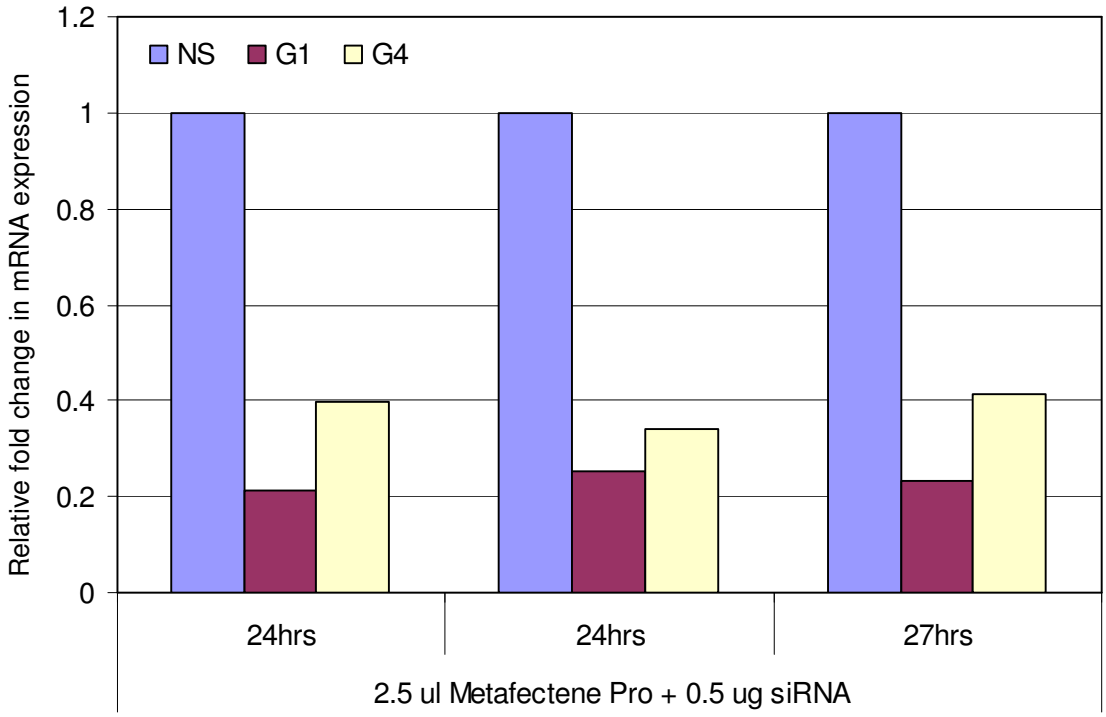

Figure 2. GFI1 protein expression measured by flow cytometry following its silencing in human primary CD8+ T cells using siRNA G1 and G4 approximately 24 hours following transfection with Metafectene Pro.

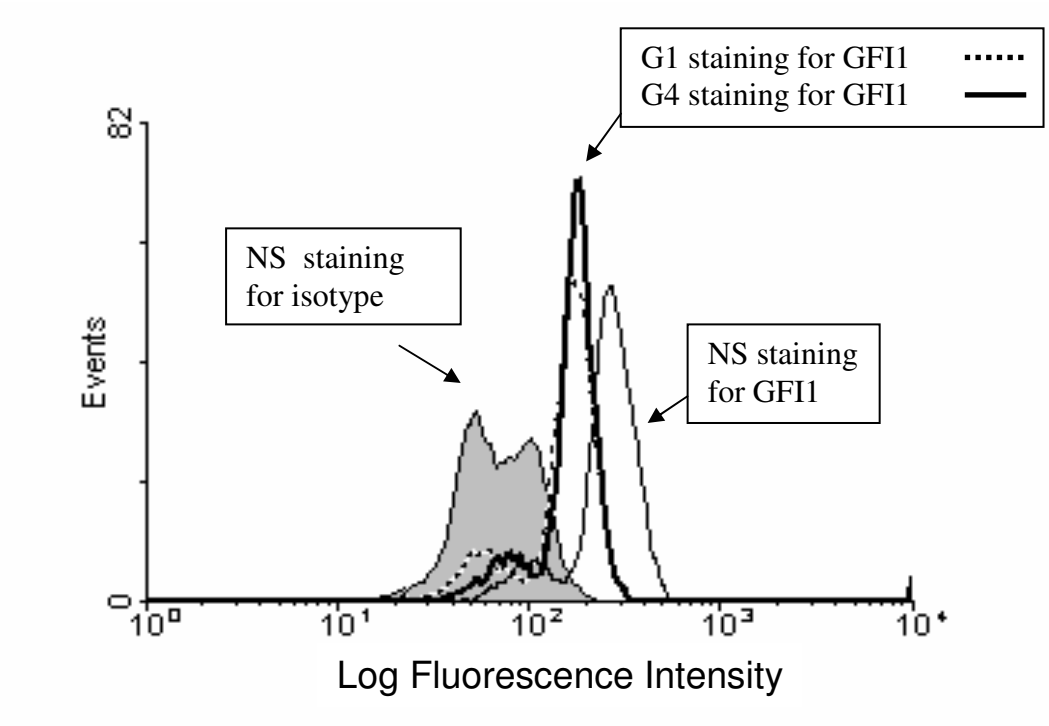

Figure 3. Average % of cell death approximately 24 hours following transfection using Metafectene Pro for each siRNA studied from the experiments in Figure 1.

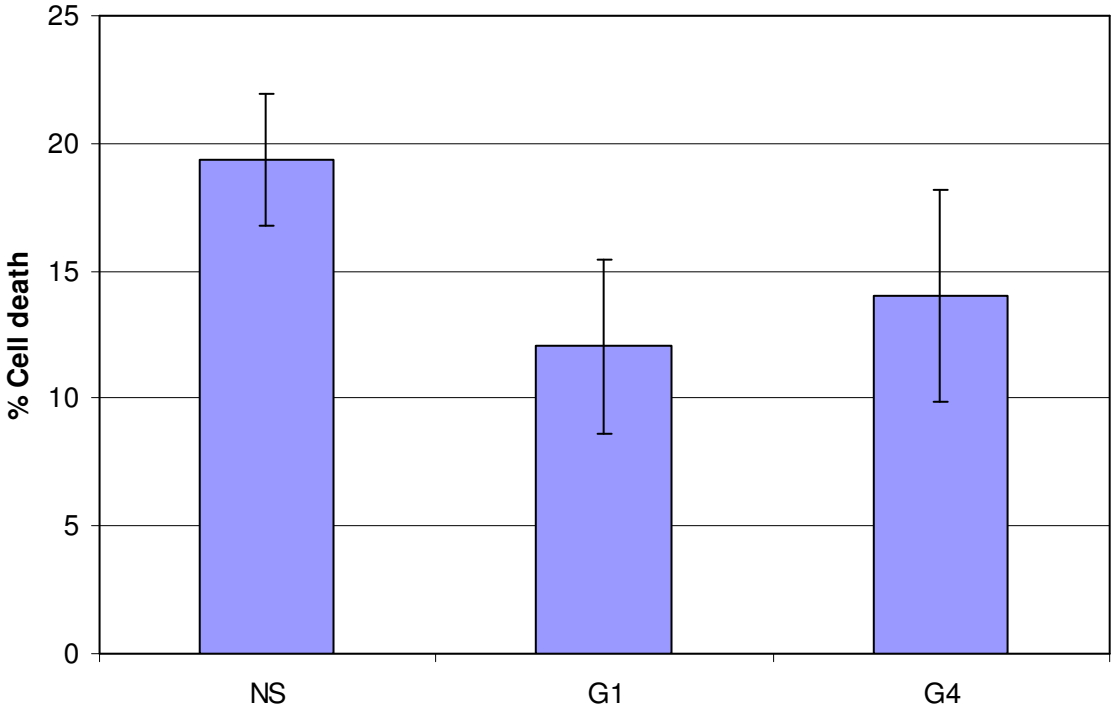

Table 1. Relative GFI1 mRNA expression levels 48 hours after transfection of CD8+ T cells with siRNA targeting GFI1 using the HiPerfect Transfection Reagent by Qiagen.

	HiPerfect								
	3 ul			6 ul			9 ul		
	siRNA (0.75 ug)								
	NS	G1	G4	NS	G1	G4	NS	G1	G4
GFI1	1		0.4165	1		1.0668	1		0.3131
siRNA (1.5 ug)									
	NS	G1	G4	NS	G1	G4	NS	G1	G4
GFI1	1	1.3073	0.6120	1	0.5560	0.3391	1	1.4372	1.2570

- Experiments with the HiPerfect Transfection Reagent were conducted as indicated in the provided instruction manual with $2x10^5$ CD8+ T cells. The experiments were conducted once and the kit provided was sufficient for the experiments above as well as for evaluating the relative gene expression levels of proteins not targeted by the siRNAs investigated. Therefore, 0.5 ug of siRNA and the additional experiments with G1 for 0.75 ug were not investigated because of the limited amount of transfection reagent. Cells were collected only at a single time point 48 hours. Values represent relative fold change in GFI1 mRNA expression.

Table 2. Cell numbers calculated 48 hours after Transfection of $2x10^5$ cells (Table 1) with HiPerfect Transfection Reagent.

	NS		G1		G4	
HiPerfect (ul)	1.5 ug	0.75 ug	1.5 ug		1.5 ug	0.75 ug
3	$1.5x10^5$	$1.2x10^5$	$1.1x10^5$		$1.4x10^5$	$1.8x10^5$
6	$1.0x10^5$	$1.3x10^5$	$1.5x10^5$		$1.5x10^5$	$1.7x10^5$
9	$1.3x10^5$	$1.7x10^5$	$1.5x10^5$		$1.5x10^5$	$1.2x10^5$