Knockdown of p53 by Accell self-delivering siRNA causes inhibition of p53-dependent DNA damage response in IMR-32 neuroblastoma cell line and β-amyloid toxicity in rat cortical neurons

Zakina Strezoska, Tamara Seredenina, Devin Leake, Annaileen Vermeulen
Dharmacon, now part of GE Healthcare, 2650 Crescent Drive, Suite #100, Lafayette, CO 80026, USA
1 Siena Biotech S.p.A., Strada del Petriccio e Belriguardo 35, 53100 Siena, Italy

Introduction

Neuroblastoma cell lines and primary neuronal cultures are commonly used as cellular model systems for studying cancer and neuronal development as well as being highly relevant models for the study of neurodegenerative diseases. However, most neuroblastoma cell lines and practically all primary neuronal cells suffer from low transfection efficiency due to the refractory nature of the cells to lipid-based transfection reagents. As such, addition of siRNA for inducing RNA interference (RNAi) has limited utility in these cell types; thus limiting their usefulness for development of functional assays for screening and discovery of novel disease-relevant genes. Dharmacon’s Accell® siRNA enables efficient delivery in a wide range of cell lines and primary cells. Accell siRNA reagents carry a novel chemical modification pattern that facilitates the delivery of siRNA without a need for transfection reagents.

To demonstrate the utility of Accell siRNA reagents in neuronal cells, the effects of the down-regulation of the tumor suppressor p53 was examined. This gene plays a pivotal role in mediating DNA damage-induced apoptosis as well as conferring a protective effect from β-amyloid peptide-induced neurotoxicity. Here we describe how application of Accell siRNA enabled the development of a high content screening assay in IMR-32 neuroblastoma cells and a whole culture cell viability assay in primary rat cortical neurons.

The ability to modulate gene expression in neuronal cell lines and primary neurons using Accell siRNA opens new opportunities for functional genomic siRNA screens in the era of neuroscience.

Experimental design


Day 0

IMR-32 Neuroblastoma cell line

Day 2

Camptothecin

Day 3

Assay for cell viability p53 and p21 induction

The cells were analyzed for cell survival by CTB assay and for induction of p53 pathway by High Content Analysis (HCA) using Thermo Scientific™ Cellomics™ Multiplexed p53 and p21 Detection Kit.

Testing media compatibility for Accell siRNA delivery in primary neurons

Primary neurons have special media requirements Neurobasal medium with B27 supplemental so Accell siRNA delivery conditions were optimized to determine the best conditions for cell viability and target gene silencing. Conditions tested:
- 100% Neurobasal medium (NBM)
- 50:50 NBM and Accell Delivery Media (ADM)
- 75:25 NBM and ADM
- 100% ADM

Optimal conditions identified: 50:50 ADM: NBM that provide maximum target silencing with high cell viability.

Efficient delivery of Accell siRNA in primary rat cortical neurons

- Confocal microscopy reveals virtually all neurons are positive for Accell GAPDH Red siRNA (A).
- Detailed analysis showed that siRNAs are localised in the cytoplasm in neuronal cell bodies and in neurites (B).
- Confluent microscopy (30x; LSM 510; Blue staining Hoechst) indicates nuclei. Scale bar 10 μm.

Neurons from E18 rats at 4 DIV (days in vitro): Accell GAPDH Red siRNA (1 μM) 48 hours.

Knockdown of p53 increases the survival of IMR-32 after camptothecin treatment

Effect of 4 μM camptothecin on IMR-32 cells transfected with different Accell siRNAs targeting p53.

Cells were treated with different CAMPOTHECIN Doses for the last 24 hours

Accell SMARTpool and two individual siRNAs targeting p53 cause a significant rescue from camptothecin-induced cell death. This is observed in the phase contrast cell images and the Promega® CellTiter Blue™ CTB cell viability assay at 72 hours post-transfection.

HCA analysis: Reduction in p53 and p21 following camptothecin treatment when p53 is silenced

A. Multiplexed p53 and p21 Detection Kit.

B. Multiplexed p53 and p21 Detection Kit.

C. Multiplexed p53 and p21 Detection Kit.

D. Multiplexed p53 and p21 Detection Kit.

Day 0: IMR32 cells transfected with different Accell siRNAs

Day 2: +/- 4 μM Camptothecin (Campt) for 20 hours

Day 3: Cells fixed and stained with Thermo Scientific™ Cellomics™ Multiplexed p53 and p21 Detection Kit.

A. Mean nuclear intensities in channel 2 (p21)
B. Mean nuclear intensities in channel 3 (p53)
C. Fluorescence in channel 1 (Hoechst stain) for p53 nuclear stain (N=500 cell)
D. Fluorescence in channel 1 (Hoechst stain) for p21 nuclear stain (N=500 cell)

Conclusions

- Silencing of p53 in IMR32 cells by Accell siRNA caused an increase in cell survival upon camptothecin treatment.
- HCA with Cellomics Multiplexed p53 and p21 Detection Kit showed a decrease in the p53 and p21 activation following camptothecin treatment in IMR-32 cells transfected with Accell siRNA targeting p53.
- Delivery of Accell siRNA was optimized in rat cortical primary neurons.
- Silencing of p53 in primary rat cortical neurons resulted in a significant increase of neuronal survival upon β-amyloid treatment.
- Accell siRNA delivery technology permits functional target validation in neuroblastoma cell lines as well as primary cortical neurons.

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