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

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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, application 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™ Accell™ siRNA enables efficient delivery in a wide range of cell lines and primary cells. Accell siRNA reagents carry a novel chemical modication pattern that facilitates the delivery of siRNA without a need for transfection reagents.

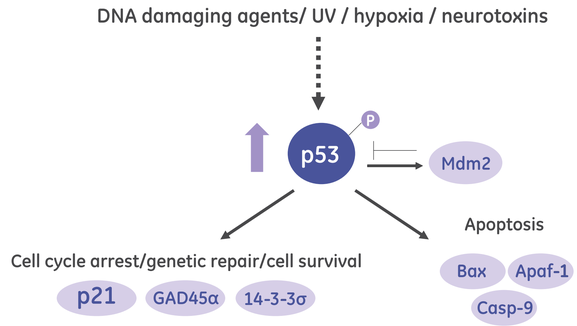
To demonstrate the utility of Accell siRNA reagents in neuronal cells, the effects of the down-regulation of the tumor suppressor *p53* was examined. This gene plays a pivotal role in mediating DNA damage-induced apoptosis as well as conferring a protective effect from β-amyloid peptide-induced neurotoxicity.

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.

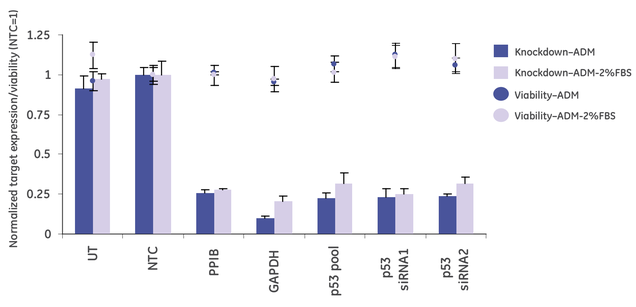
The ability to modulate gene expression in neuronal cell lines and primary neurons using Accell siRNA opens new opportunities for functional genomic siRNA screens in the eld of neuroscience.

p53 is a mediator of numerous cell-damaging agents

Cell exposure to damaging agents induces rapid increase of p53 protein levels in the nucleus, leading to the induction of its transcription targets which control cellular responses such as cell cycle arrest, repair and apoptosis.



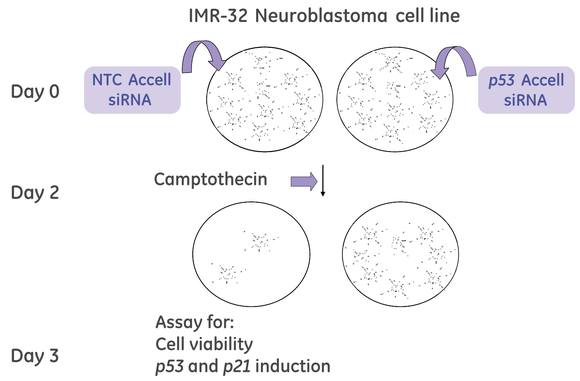
Efficient target mRNA knockdown in IMR-32 cells by Accell siRNA



- IMR32 cells at 20 000 cells/well on Collagen IV plates
- 1 µM Accell siRNAs control pools (NTC, *PPIB* or *GAPD*) and Accell SMARTpool or individual siRNAs against *p53* delivered in Accell Delivery Media (ADM) with or without addition of 2% serum
- Knockdown and viability assessed at 72 hours post-transfection
- UT = untreated with siRNA; NTC = Non-targeting control pool

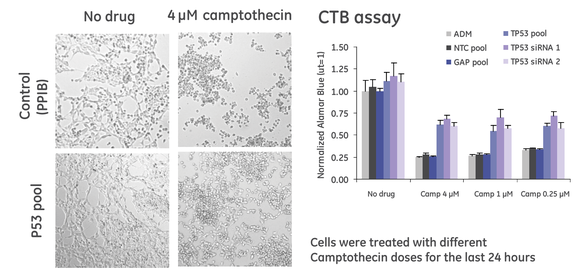
Experimental design

Purpose: Determine if knockdown of *p53* by Accell siRNA will cause suppression of the p53-dependent DNA damage response pathway in IMR-32 cells upon camptothecin treatment.



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.

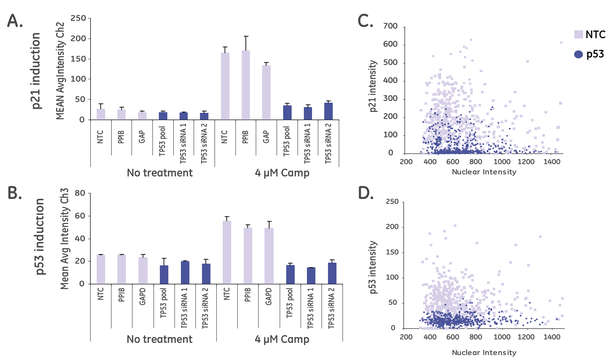
Knockdown of *p53* increases the survival of IMR-32 after camptothecin treatment



Cells were treated with different Camptothecin doses for the last 24 hours

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

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



Day 0: IMR32 cells transfected with different Accell siRNAs

Day 2: -/+ 4 µM Camptothecin (Camp) 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. Intensity in channel 1 (Hoechst stain) vs the intensity in channel 2 for p21 nuclear stain (N=500 cell)
D. Intensity in channel 1 (Hoechst stain) vs the intensity in channel 3 for p53 nuclear stain (N=500 cell)

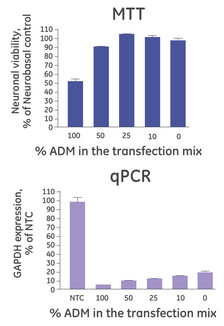
Testing media compatibility for Accell siRNA delivery in primary neurons

Primary neurons have special media requirements (Neurobasal medium with B27 supplements) 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 identied (50: 50 ADM: NBM) that provide maximum target silencing with high cell viability.

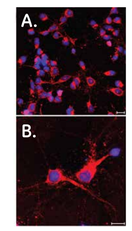


Efficient delivery of Accell siRNA in primary rat cortical neurons

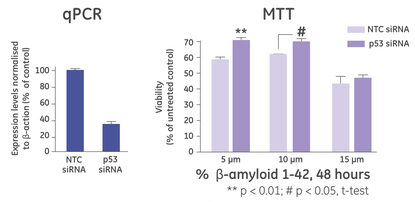
- Confocal microscopy reveals virtually all neurons are positive for Accell GAPD Red siRNA (A).
- Detailed analysis showed that siRNAs are localised in the cytoplasm in neuronal cell bodies and in neurites (B).

Confocal microscopy (Zeiss 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.



p53 knockdown provides neuroprotective effect from β-amyloid peptide in primary rat cortical neurons



** p < 0.01; # p < 0.05, t-test

- More than 60% down-regulation of *p53* mRNA at 48 hours post-transfection by Accell SMARTpool reagent.
- Silencing of *p53* resulted in a signicant increase of neuronal survival as compared to NTC siRNA as measured by MTT viability assay.
- The strongest protective effect was observed at 5 µM β-amyloid.

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 signicant 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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