

Effects of Thermo Scientific Dharmacon® SMARTvector™ shRNA Lentiviral Particles transduced into the neuroblastoma cell line

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Introduction

The neuroblastoma cell line SH-SY5Y was used to demonstrate the ability of the SMARTvector shRNA lentiviral technology to sequence specifically silence genes of interest. The SH-SY5Y cell line is a mixed population of neuronal and endothelial cells which differentiates in response to all-trans retinoic acid. The study's goal was focused on identifying factors that promote neural development, inhibit neural outgrowth and factors that can overcome this inhibition. Genes targeted for this study included RAC1 which promotes neurite outgrowth and RhoA that inhibits neurite outgrowth (Figure 1). Once transduced and fluorescently labeled, images and data were collected using a high content screening approach to quantify neurite outgrowth measurements including neurite count, neurite average length and branch point average count.

Materials and Methods

The SH-SY5Y cells were obtained from ATCC (Manassas, VA) and transduced with the shRNA silencing constructs targeting RAC1 and RhoA using the SMARTvector shRNA lentiviral technology. The cells were then treated with alltrans retinoic acid (ATRA), or left untreated (UT) for 9 days. After fixation, the cells were stained with the Thermo Scientific Cellomics® Neurite Outgrowth HCS Reagent Kit; images were acquired on the Thermo Scientific Cellomics ArrayScan® VTI HCS Reader and analyzed with Thermo Scientific Cellomics Neuronal Profiling BioApplication (Therm Fisher Scientific, Pittsburgh PA).

Results

The mixed population of neuronal and endothelial cells was separated for analysis using the gating techniques available on the Cellomics ArrayScan VTI using the Neuronal Profiling BioApplication (Figure 2). ATRA

induces neurite outgrowth in a dose dependent manner. Compared to untreated cells, ATRA increases neurite outgrowth with 10 μ M as a maximal dose (Figure 3). The product of the RhoA gene is known to inhibit neurite outgrowth. When the cells were transduced with the RhoA shRNA SMARTvector shRNA lentiviral particles, depletion of RhoA resulted in an increase in neurite outgrowth compared to the SMARTvector Non-targeting control (NTC) particles (Figures 4 A and C and Figure 5). Cells that were transduced with the Rac1 SMARTvector shRNA lentiviral particles showed a slight inhibition of neurite outgrowth (Figure 5). The product of the RAC1 gene is known to promote neurite outgrowth. When the RhoA and RAC1 transduced cells were treated with ATRA, the knockdown of RAC1 inhibited neurite outgrowth compared to the SMARTvector NTC particles (Figures 4 B and D and Figure 6) while knockdown of RhoA showed no additional effect on neurite outgrowth (Figure 6).

アプリケーション
ノート:
LC01662000

RNAi

- ArrayScanVTI
- Neuronal Profiling BioApplication
- HCS蛍光試薬キット

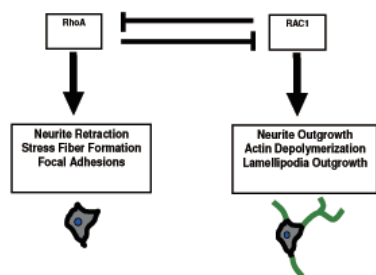


Figure 1. General Effects of RhoGTPases on Neurite Outgrowth. RhoGTPases: RAC1 and RhoA affect neuronal outgrowth by regulating the actin cytoskeleton. RAC1 activates its downstream effectors to stimulate neurite outgrowth. RhoA inhibits neurite outgrowth by activation of its downstream effectors.

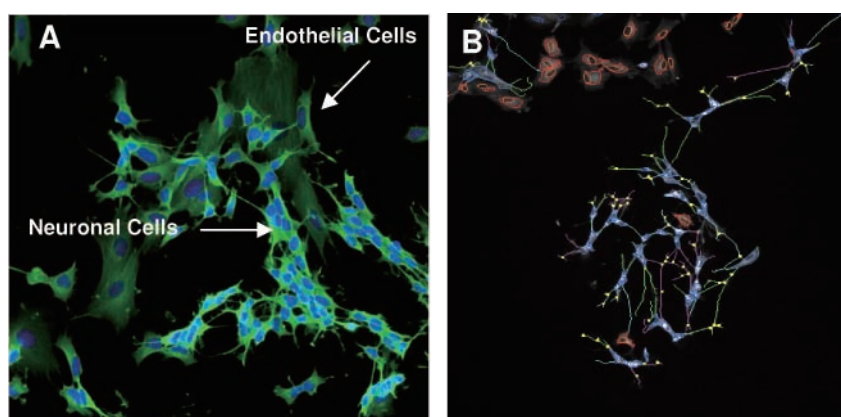


Figure 2. Analyzing the mixed population of neuronal and endothelial cells in the SH-SY5Y cell line. (A) Composite image of the SH-SY5Y cell line where the green represents the neuronal bodies and the endothelial cells at different intensities. (B) Raw image with algorithmic overlays from the Cellomics Neuronal Profiling BioApplication indicating the endothelial cells (orange demarcation) that were removed from the analysis based on their cell body average intensity. Other overlays indicate cell body and neurite demarcation.

Neurite Outgrowth Assay
SHSY5Y 2K-well 6 Days
Treated with DMSO or All-Trans Retinoic Acid
Normalized to DMSO

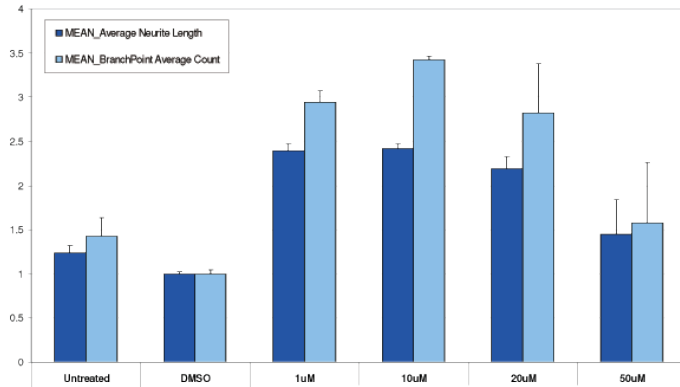


Figure 3. Effect of All-Trans Retinoic Acid on Neurite Length and Amount of Neurite Branching. SH-SY5Y cells were treated with increasing concentrations of ATRA.

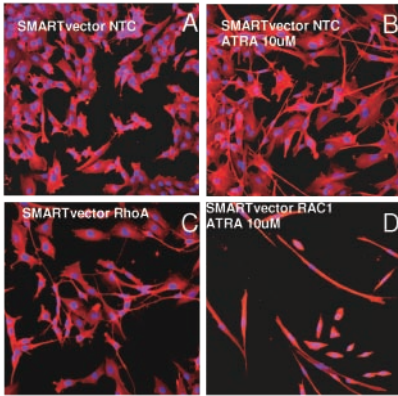


Figure 4 Results of gene depletion on neurite outgrowth. (A) SMARTvector non-targeting control (NTC) particles (B) SMARTvector NTC particles treated with ATRA (10 μM) stimulates neurite outgrowth (C) RhoA knockdown enhances in neurite outgrowth in untreated SH-SY5Y cells (D) RAC1 knockdown inhibits neurite outgrowth in the presence of ATRA.

Non-Selected SMARTvector Transduced SHSY5Y
Neurite Outgrowth Assay 9 Days Post-Transduction
Cultured in Growth Media Only
Normalized to SMARTvector NTC

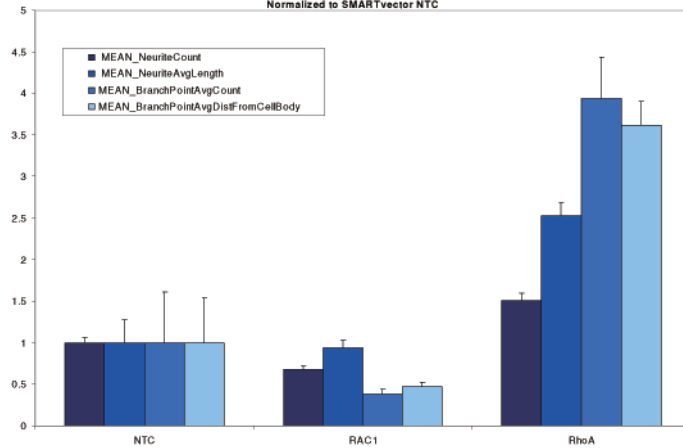


Figure 5. Depletion of RhoA stimulates neurite outgrowth and depletion of RAC1 inhibits neurite outgrowth when normalized to non-targeting control.

Non-Selected SMARTvector Transduced SHSY5Y
Neurite Outgrowth Assay 9 Days Post-Transduction
Cultured in Retinoic Acid 10μM
Normalized to SMARTvector NTC

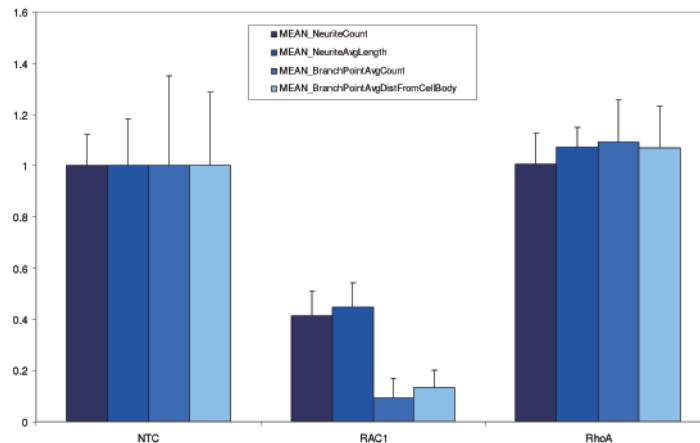


Figure 6. Depletion of RAC1 inhibits neurite outgrowth in the presence of ATRA while depletion of RhoA did not increase neurite outgrowth in the presence of ATRA when normalized to ATRA treated SMARTvector NTC particles.

Conclusions

- The Dharmacon SMARTvector shRNA lentiviral technology successfully transduced the difficult-to-transfect neuroblastoma cell line, SH-SY5Y.
- The Celloomics ArrayScan VTI HCS Reader and the Neuronal Profiling BioApplication software enabled the analysis of only the targeted cells in a mixed population and identified the effects of silencing genes involved in neurite outgrowth.
- The Dharmacon SMARTvector shRNA lentiviral knockdown experiments were quantitated with the Neuronal Profiling BioApplication illustrating the utility of combining these two powerful technologies.

References

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 C. Dey, N, et al. (June 2007) Mol Cell Bio. 27(11): 4179-97.

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