Cellular Uptake and Intracellular Transport of Viral and Non-viral Gene Vectors in Differentiated Neurons Affected in Parkinson's Disease

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Neurodegenerative disorders, such as Parkinson's Disease (PD) and Alzheimer's Disease, are mainly due to genetic mutations of differentiated neurons that lead to protein malfunction. Thus, therapeutic gene delivery to differentiated neurons is an attractive strategy to combat these diseases. Unfortunately, gene delivery into fully differentiated neurons is significantly less efficient than delivery into actively dividing undifferentiated cells. Furthermore, non-viral gene vectors suffer from lower transfection efficiencies compared to viral vectors.

To uncover mechanistic reasons for these observations, we used flow cytometry and real-time Confocal Particle Tracking (CPT) to elucidate the contribution of cellular uptake, endosomal escape, and cytoplasmic transport to the overall gene delivery process. We studied the dopaminergic SH-SY5Y neuroblastoma cell line that can be induced to differentiate into post-mitotic neuron-like cells. This cell type allows us to quantitatively investigate the differences in gene delivery between undifferentiated (transfection permissive) and differentiated (transfection restrictive) neurons. To investigate the discrepancy in intracellular transport and trafficking between non-viral and viral gene vectors, fully differentiated primary rat cortical neurons were studied.

Undifferentiated SH-SY5Y cells were transfected over 50-fold greater than differentiated neurons with optimized PEI/DNA nanocomplexes. To determine if cellular uptake may be a critical barrier to gene delivery into differentiated neurons, we used flow cytometry to measure the percentage of cells with internalized PEI/DNA nanocomplexes. Differentiated neurons displayed 2-fold lower internalization of fluorescently labeled gene vectors than undifferentiated cells. Therefore, differences in cellular uptake may contribute to the difference in gene transfection between differentiated and undifferentiated neurons. Methods to improve cellular uptake of non-viral gene vectors, such as attaching bioactive ligands to the vector surfaces, is currently being pursued.

Next, CPT was used to quantitatively compare the intracellular transport and trafficking of low efficiency non-viral vectors (PEI/DNA) to high efficiency viral vectors (adenovirus 5) in fully differentiated primary cortical neurons. 20s movies of gene vectors were captured at 20 frames per second and analyzed with MetaMorph software to obtain individual particle mean-square displacements (MSD) and effective diffusivities. Most internalized PEI/DNA nanocomplexes and adenoviruses were found in the perinuclear region of rat cortical neurons after 1h post-transfection. Both types of gene vectors displayed diffusive or anomalous sub-diffusive transport in the primary neurons. A minority of both PEI/DNA nanocomplexes and adenoviruses underwent active transport. Interestingly, a majority of PEI/DNA nanocomplexes co-localized with acidic vesicles, but co-localization was not observed for adenoviruses. PEI/DNA nanocomplexes, therefore, may be sequestered in intracellular vesicles, whereas adenoviruses may efficiently escape the vesicles. Thus, efficient endosomal escape may be a key feature of viral vectors that allow them to deliver genes into differentiated neurons more effectively. Attachment of endosomolytic agents to non-viral vectors may be required to improve transfection efficiency in differentiated neurons.

Mechanistic studies of cellular uptake and intracellular trafficking of non-viral PEI/DNA nanocomplexes reveal these gene vectors suffer from sub-optimal cellular uptake rates and adverse trafficking in fully differentiated neurons. Methods to overcome these barriers must be exploited to improve overall gene delivery efficiency for non-viral gene therapy for neurodegenerative diseases.