Tracking the Intracellular Path of Fluorescently Labeled DNA Delivered by PEI Nanocomplexes in Live Cells

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Polyethylenimine (PEI)/DNA nanocomplexes are actively transported to the perinuclear region of live cells (1). Recent results from our lab show PEI/DNA nanocomplexes are actively transported in vesicles of the endo-lysosomal pathway, eventually resulting in the accumulation of gene vectors in perinuclear lysosomal compartments (2).

In these studies (1,2), the intracellular transport of PEI/DNA nanocomplexes was tracked by fluorescently labeling the PEI component of the gene vectors. To determine if the DNA component of PEI/DNA complexes also follow the same intracellular route, we fluorescently labeled DNA in the complexes with an intercalating dye, POPO-3 (Molecular Probes). The intracellular transport of these gene vectors in live HeLa cells was characterized, both qualitatively and quantitatively, with Real-time Confocal Particle Tracking (CPT) (2). At early times post-transfection, the intracellular transport rates of gene vectors tracked by fluorescently labeling DNA were similar to gene vectors tracked by fluorescently labeling PEI.

Approximately 30% of HeLa cells were successfully transfected with PEI/DNA nanocomplexes. We sought to determine the location of the delivered DNA in positively transfected cells only, hoping to uncover the key differences in DNA trafficking between transfected cells and those that were not successfully transfected. To accomplish this, PEI/DNA nanocomplexes were formed with plasmids encoding the green fluorescent protein (GFP). The plasmids themselves were fluorescently labeled with POPO-3, and the intracellular location of fluorescently labeled DNA was compared in cells expressing or not expressing GFP.

At 24 h post-transfection, the signal from fluorescently labeled DNA was rarely detectable in positively transfected cells and, therefore, few PEI/DNA nanocomplexes were visible. In contrast, cells that were not transfection exhibited significant intracellular accumulation of fluorescent PEI/DNA complexes, visible due to the fluorescently labeled DNA. These results suggest that DNA is dissociated from PEI in positively transfected cells, resulting in faint diffuse fluorescence signals indistinguishable from cellular auto-fluorescence. Dissociation of DNA from the PEI may have occurred after endosomal escape of the gene vectors. Also, the POPO-3 dye may have dissociated from the free plasmid DNA, especially if the plasmid DNA was delivered into the nucleus where there exists a large concentration of chromosomal DNA that can compete for the fluorescent dye. Methods to covalently attach fluorescent dye molecules to plasmid DNA are currently being pursued to overcome the limitations of POPO-3 dye and to continue the mechanistic investigation of the intracellular gene delivery process.

1. Suh, J., J. Hanes, *Efficient active transport of gene nanocarriers to the cell nucleus*. PNAS, 2003, 100: 3878-3882.

2. Suh, J., Y. An, B. Tang, J.S. Suk, J. Hanes, *Real-time correlation of intracellular gene vector transport rates with biological location in live Mesenchymal Stem Cells*. Proceedings of the American Institute of Chemical Engineers, 2004.