

Real-time Correlation of Intracellular Gene Vector Transport Rates with Biological Location in Live Mesenchymal Stem Cells

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Gene delivery to Mesenchymal stem cells (MSCs) has many potential clinical and tissue engineering applications. One of the most effective non-viral gene vectors used currently is polyethylenimine (PEI), owed to its ability to condense DNA into small nanoparticles and to potentially escape endosomes by disrupting vesicle membranes. Unfortunately, the intracellular trafficking of PEI/DNA nanocomplexes in MSCs is poorly understood, thereby limiting our knowledge of the gene delivery process and making the rational evolution of gene vectors difficult.

Non-viral gene vectors are capable of active transport in cells along microtubules to the perinuclear region in less than 30 minutes, a mechanism similar to several of nature's efficient viral vectors. It is unclear, however, whether gene vectors are actively transported within endosomes or if they must first escape the vesicles. This question is significant since rapid endosomal escape has typically been desired, but may in fact limit delivery efficiency if transport through endosomes is required for efficient transport through the cell cytoplasm to the perinuclear region.

To directly correlate active gene vector transport with biological location (i.e. intracellular vesicles), we developed multi-color Confocal Particle Tracking (CPT). This method allows us to correlate quantitative intracellular gene vector transport rates to biological location in live cells and in real time. We use a high-speed confocal microscope (Zeiss LSM 510 Meta) with the ability to capture fluorescence signals from multiple different fluorophores with the same detector (i.e. Meta detector). The signals from the different fluorophores are then separated by emission fingerprinting, allowing for the simultaneous detection of multiple color species (e.g. gene vectors and organelles) at fast capture rates (as fast as 20 frames per second). Multi-color CPT allows for characterizing the transport of gene vectors in live cells in four dimensions: x, y, time, and color (biological location).

MSCs were first transfected with genes encoding fluorescent marker proteins for specific organelles: EEA1-GFP for early endosomes (EE) or Niemann Pick C1 (NPC1)-RFP for late endosomes/lysosomes (LE/Lys). This resulted in the fluorescent labeling of EE or LE/Lys and allowed for live-cell co-localization studies with gene vectors labeled with a different fluorophore. Fluorescently labeled PEI/DNA nanocomplexes were added to cells and their intracellular transport and trafficking were investigated with CPT. To our knowledge, this is the first time the intracellular transport of gene vectors have been quantified and directly correlated to biological location in real time. We can now directly link the transport rate of a gene vector to the biological compartment in which the gene vector resides, helping to elucidate the dynamic mechanisms of intracellular gene delivery.

At early times post-transfection, PEI/DNA nanocomplexes co-localized with the marker for EE. The transport properties of gene vectors in EE were regulated by the motion of the early endosomes. Remarkably, we were able to capture a small fraction of

gene vectors leaving EE in real time, possibly escaping the vesicles or entering LE/Lys. Over time post-transfection, increasing amounts of PEI/DNA complexes were found in LE/Lys, organelles that display perinuclear distribution. Over 80% of gene vectors were in perinuclear LE/Lys compartments by 32 h post-transfection. A small fraction of gene vectors were seen entering LE/Lys in real time, possibly coming from EE vesicles. Majority of gene vectors in EE or LE/Lys displayed anomalous sub-diffusive transport, resulting in minimal displacements from their initial positions. A minority of the vectors in vesicles, however, did exhibit active transport characterized by linear/curvilinear trajectories that were often saltatory (stop-and-go motion).

Multi-color Confocal Particle Tracking is a new quantitative tool that allows improved correlation of biology with physical phenomena in live cells. Studies using this technology show the characteristic intracellular transport of PEI/DNA nanocomplexes in MSCs is dictated by the transport properties of early endosomes and late endosomes/lysosomes through which the gene vectors are trafficked. This trafficking leads to the efficient transport of gene vectors to the perinuclear region and a persistently high local concentration of the vectors close to the nucleus. Gene vectors designed to escape endosomes efficiently must consider the kinetics of endo/lysosomal trafficking, since premature endosomal release may prevent highly efficient transport to the nuclear region. Methods for gene vectors to specifically penetrate the membranes of the perinuclear late endosome/lysosomes may significantly improve the efficiency of intracellular gene delivery into Mesenchymal stem cells.