Immobilization of Gene Vectors in Perinuclear Region as Potential Intracellular Barrier to Efficient Gene Delivery

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Non-viral polyethylenimine (PEI)/DNA nanocomplexes were shown to be actively transported along microtubules from the cell periphery to the perinuclear region as efficiently as viral vectors, disproving the common belief non-viral vectors are less efficient due to their slow, random movement through the vast cytoplasm. Therefore, critical intracellular barriers to gene delivery with this vector were narrowed to endosomal escape, nuclear uptake, and vector unpacking. Here, we suggest another potential barrier to efficient gene delivery: the immobilization of gene vectors in the perinuclear region of cells, preventing the diffusion of gene vectors to the nuclear pore complex for uptake into the nucleus.

To study the highly dynamic process of intracellular gene delivery, we used real-time multiple particle tracking (MPT) to quantify the transport of gene vectors in live cells. Briefly, the movement of fluorescently labeled polyethylenimine (PEI)/DNA nanocomplexes, an "efficient" synthetic vector, was captured with a camera attached to an epifluorescence microscope. Using MetaMorph software, vectors were tracked to obtain time-resolved positional data, which was used to calculate mean-square displacements, MSD. Individual vector transport parameters, such as diffusivity and velocity, were obtained from the MSDs.

After reaching the perinuclear region rapidly (within 30 minutes), most gene vectors appeared immobile. A fraction of the immobilized gene vectors displayed restricted "pearls-on-a-string" trajectory, which may reflect binding/unbinding of vectors to microtubules or other structures. Whether these vectors are inside (pre-escape) or outside (post-escape) endocytic vesicles, is unclear. Endosomes have on/off kinetics with microtubules; therefore, vectors within endosomes may reflect endosome transport properties.

Interestingly, PEI/DNA nanocomplexes alone (i.e. outside of endosomal vesicles) displayed strong attraction to purified microtubules in an *in vitro* motility assay, suggesting vectors post endosome escape may also associate with cytoskeletal elements to display "pearls-on-a-string" trajectories, resulting in poor displacements. Therefore, vectors that actively transport to the perinuclear region, and successfully escape vesicles, may still face a formidable task in delivering their cargo into the nucleus.

These results indicate a possible need for preventing unwanted binding of drug/gene vectors to intracellular components, thereby improving the ability of vectors to reach the nuclear envelope. Indeed, surface modification of drug/gene vectors with the hydrophilic polymer, polyethylene glycol (PEG), enhanced their intracellular transport rates (1). Novel insights revealed with particle tracking, such as those presented here, should help guide the rational evolution of gene vectors into highly efficient delivery systems.

1. Choy, K., J. Suh, J. Hanes, *PEG Improves Intracellular Transport of Drug/gene Carriers as Revealed by Real-Time Particle Tracking*. Proceedings of the American Institute of Chemical Engineers, 2004.