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Design of hybrid synthetic retroviral gene delivery vectors

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Abstract

Gene therapy has the potential to revolutionize healthcare for millions of people. However, it has yet to become a common treatment for the variety of diseases that could benefit from the delivery of therapeutic genes. The current implementation of gene therapy must be preceded by the development of vectors with improved characteristics.

We have developed novel and improved gene therapy vectors comprising of synthetic polymer-based – chitosan (χ) and lipid-based (φ) envelopes for Moloney Murine Leukemia Virus (MLV) -like particles (M-VLPs). M-VLPs are essentially intact viruses lacking the envelope protein most necessary for transfection, thus making them inactive. Both chitosan and liposomes composed of DOTAP, DOPE and cholesterol electrostatically associated with M-VLPs forming hybrid vectors that could deliver M-VLPs without the need for the biological envelope protein. The transfection efficiency of these hybrid vectors (χ/M-VLPs and φ/M-VLPs) was not only better than our earlier hybrid vectors (PEI/M-VLPs and PLL/M-VLPs) but also of the same order of magnitude as amphotropic MLVs (MLV-A).

Uptake of χ/M-VLPs was primarily via endocytic pathways but intracellular trafficking was dependent on the pH of the chitosan used for forming the hybrid vectors. χpH3/M-VLPs were dependent on clathrin-mediated endocytosis whereas χpH4/M-VLPs were caveolae mediated with macropinocytosis playing a minor role. Cellular uptake of φ/M-VLPs was dependent on DOTAP content with high DOTAP mediating higher cell entry although successful transfections were not dependent on total uptake levels. Uptake of φ/M-VLPs was via both endocytosis as well as passive fusogenicity with the plasma membrane. However, successful gene delivery required an endocytic pathway. Intracellular trafficking of φ/M-VLPs was dependent on the lipid composition with a high presence of DOPE being clathrin-dependent and high cholesterol content being caveolae mediated. φ/M-VLPs also had significantly faster trafficking kinetics as compared to χ/M-VLPs but slower than MLV-A which was confirmed by inhibition of reverse transcription and visualization via confocal microscopy.

It can be concluded that the synthetic component of the hybrid vectors not only allows for a modified trafficking mechanism of the retroviral particle but also modulates the kinetics of delivery of the retrovirus to the nucleus for efficient expression of the target gene.

Biography

Rahul K. Keswani is a Ph.D. in Chemical and Biomolecular Engineering from the University of Illinois, Urbana-Champaign where his research focus was the development and characterization of retroviral vectors for human gene therapy. He obtained his B.Tech. in Chemical Engineering from the Indian Institute of Technology, Bombay where he studied the synthesis of titania nano-particles via microemulsions. At Imperial College, UK, he studied the characterization and novel design of polymer-glass scaffolds for tissue engineering applications. Currently, he is working as a Senior Process Engineer at Orochem Technologies Inc., researching continuous chromatography methodologies for the purification of pharmaceuticals and biomolecules.