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Design of hybrid synthetic retroviral gene delivery vectors

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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.

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. Limited progress is primarily due to the lack of a safe and efficient means of delivering genetic material. Viral vectors, for instance, are extremely efficient but potentially pathogenic and immunogenic. They also typically possess a tropism towards specific cellular receptors which is difficult to modify without significant loss in efficiency. Non-viral vectors are typically non-pathogenic and nonimmunogenic, yet lack the efficiency necessary for gene therapy and are also typically toxic at clinically useful concentrations. χ/M-VLP suffered from high precipitation issues when stored at 4 °C for > 4 days but exhibited better transfection efficiency than MLV-A in the same testing period. Both serum resistance and storage stability were dependent on χ/M-VLPs stoichiometry. 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. Transfection efficiency of φ/M-VLPs was dependent on the lipid composition used for the synthetic lipid envelope. Liposomes with low DOTAP, low DOPE and high cholesterol content were able to mediate better transfections with M-VLPs. 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. Overall, φ325/M-VLPs was one of the most optimal synthetic lipid compositions in terms of transfection efficiency. The lipid toxicity was also dependent on the composition of the lipid envelope with higher cholesterol content leading to low toxicity. Significant size reduction of the φ/M-VLPs was achieved via liposomal extrusion prior to association with M-VLPs. φ/M-VLPs were able to provide stable transgene expression over a period of three weeks but exhibited poor serum stability. The storage stability of φ/M-VLPs was significantly better than χ/M-VLPs and MLV-A in terms of transfection efficiency and vector size. 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.

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