25b Engineering Enhanced Retroviral Vectors for Gene Therapy through the Generation and Selection of 6xhis Peptide Insertion Libraries into Vsv-G

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Engineering of retroviral vectors by inserting novel protein or peptide sequences into viral proteins could readily improve their properties as gene therapy vehicles. Enhancements to VSV-G, an envelope protein commonly used to pseudotype retroviral and lentiviral vectors, could aid in vector purification and cell-specific targeting. However, because the structure of VSV-G is unknown, it has been difficult to rationally determine where to insert a new sequence without disrupting the virus' ability to transduce cells. We have developed a library approach to modify viral genomes through the random insertion of peptides that may confer novel functions to the viral vector. This high throughput method was used to generate a 24,000 member library of VSV-G mutants that have a 6xHis tag randomly incorporated which indicates comprehensive insertion at all possible points in the original 1.5 kilobase gene sequence. Current methods of purifying retroviral vectors for clinical applications retain protein and packaging cell genomic DNA contaminants that pose a safety and immune response risk in a patient. Affinity chromatography offers a scalable and economical process that can generate clinical grade preparations while recovering a high yield of active vector. We have selected the VSV-G-6xHis library to isolate variants that can be purified by immobilized metal affinity chromatography (IMAC) while still retaining their ability to transduce cells. Purification of vectors pseudotyped with these novel clones by IMAC resulted in yields comparable to current protocols; however, it dramatically reduced the levels of contaminating protein and DNA over 1400-fold and 6000-fold, respectively, representing a major improvement. Furthermore, column-purified lentiviral vectors pseudotyped with these VSV-G-6xHis mutants have the same or better levels of expression than wild-type in vivo. In addition, we are utilizing these mutants to identify possible sites for the introduction of other desirable improvements to vectors such as cell-specific targeting, antibody evasion, and vector trafficking.