25d Enhanced Stability of Retrovirus by Directed Evolution

Halong Vu and Daniel W. Pack

Viruses are highly efficient gene transfer agents, but obviously have not been optimized for use as therapeutics or even laboratory reagents. For example, viruses have limited target cell specificities that, most often, do not match the needs of gene therapy applications. In addition, recombinant viruses are difficult and expensive to produce and purify. The infectivity half-life of a typical murine leukemia virus (MLV) preparation is only 5-8 hours at 37 C, which limits the virus production. The loss of infectivity is believed to be the result of shedding of the virus' envelope protein, which is responsible for initiating infection of target cells. We used directed evolution to generate an MLV variant that exhibits at least double the half-life of the wild-type virus through a single round of virus mutagenesis and selection. Surprisingly, however, the mutations responsible for the enhanced stability were found in the gag-pol gene, not the envelope. This presentation will describe our mutagenesis and selection strategy, characterization of the variant virus, and the mechanisms by which the improved phenotype was achieved.