

ABSTRACT

The method of directed evolution represents a new approach to generate proteins with new or altered properties. The principle of directed evolution is random mutagenesis of the coding sequence for a protein of our interest followed by selection of generated mutants for the desired property. The aim of this pilot study was to investigate the possibility of utilization of directed evolution for alteration of mouse polyomavirus original tropism and virus retargeting to a model prostate cancer cell line. To generate randomly mutated gene encoding the major capsid protein of mouse polyomavirus, which is responsible for the interaction of the virus with cellular receptor for viral cell entry, error-prone PCR and DNA shuffling methods were used. Production of viruses composed of mutant major capsid protein was ensured by Cre/loxP site-specific recombination. The thesis also dealt with the design and characterization of the system for viral mutant selection. It was found that the prostate cancer cell lines markedly vary in their ability to bind and internalize particles derived from mouse polyomavirus. This knowledge can be used for the preparation of virus-like particles for prostate cancer diagnostics in the future. The study demonstrated that the method of directed evolution can be used for production of mutant polyomaviruses and identified several problematic parts of the procedure.