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Construction and Characterization of Replication-Deficient and Cancer-Specific Replication-Competent Feline Foamy Virus Vectors

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Foamy viruses (FV) are promising candidates for the development of gene therapy vectors. This thesis focused on the development and characterization of Feline FV (FFV)-based replication-deficient (RD) and conditional replication-competent (RC) vectors for human cancer gene therapy.

Using optimized vector preparation procedures, RD FFV vector titers above 10^7 TU/ml were reproducibly obtained. The stability of transgene expression is an intrinsic feature of FFV RD vectors but is clearly influenced by the transgene used. Furthermore, RD FFV SIN vectors have intrinsically improved vector safety features regarding readthrough into flanking cellular sequences due to the abolished promoter function of the proviral SIN-LTR.

Site-directed mutagenesis studies revealed that the authentic major splice donor site is essential for efficient RD FFV vector production, most probably by regulating RNA export and cytoplasmic stability. Additionally, inactivation of the *gag* start codon abrogated expressions of truncated Gag or Gag-Pol fusion proteins and thus increased the production efficiency of novel gutless vectors. Furthermore, through refined mapping of FFV CAS II, a minimized gutless vector with 3.8 kb viral sequences was obtained with an insertional capacity up to 8 kb.

In vitro transduction assays showed that different human cancer cells are permissive toward transductions by RD FFV vectors. FFV replication in human cancer cells was self-limiting but still more efficient than non-transformed cells. Thus FFV-based RC cancer therapy vectors may have lower risks of systematic spread in human. As an approach to enhance cancer cell specificity, the wt LTR was replaced by a hybrid hCox-LTR promoter. As expected, the hybrid hCox-FFV vectors showed impaired replication capacity in feline cells but allowed low-level replication in the transduced human cancer cells.

FFV-based RD and cancer-specific RC vectors are interesting tools for gene transfer and cancer therapy respectively. Further studies are required to improve and characterize the anti-cancer efficacy of hCox-FFV RC vectors in animal models.