RNA-VIRUS WITH NONCYTOPATHIC REPLICATION - NOVEL VECTOR FOR EPIGENETIC REPROGRAMMING

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Introduction: Efficiency of obtaining of induced pluripotent stem cells (IPSCs) depends critically on the technique used to deliver reprogramming factors (transcription factors such as the Sox2, OCT4, Klf1, c-Myc). Currently the most effective systems for the production of IPSCs utilize viral vectors. Alphaviruses (gen. Alphavirus, fam. Togaviridae) comprise a genus of RNA-viruses that provide high levels of proteins from genes cloned into the viral genome, and the alphaviruses do not alter the genome of the host cell. IPSCs obtained using the Alphavirus vectors syngeneic with the donor and suitable for medical use. A cDNA of the full-length genome of a model alphavirus, the Venezuelan equine encephalitis virus (VEE) was constructed. This cDNA was used to rescue the virus from the cDNA copy. Mutations were introduced into the cDNA to make the replication noncytopathic. Mutant virus has a stable noncytopathic phenotype, grows to high titers and provides synthesis of recombinant proteins.

Methods: cDNA VEE strain TC-83 was constructed from 9 DNA fragments; each fragment was produced *de novo,* by synthesis from oligonucleotides. Viral RNA was synthesized *in vitro* and transfected into cell cultures (BHK-21, CHO, HEK293). Site-directed mutagenesis and cloning of foreign genes into the cDNA was done by PCR-mediated genetic engineering. Virus titers were determined using the Reed-Muench method.

Results: Wild-type VEE (VEEwt) was found to be cytopathic. 100% death of an infected monolayer occurred within 48 hours upon infection. Mutations were introduced into the VEE genes for non-structural protein nsP2 and the capsid protein. Mutant virus (VEEmut) exerts no cytopathicity and accumulates to high titers (>10 FFU/ml). Genes of heterologous proteins (GFP, puromycin acetyltransferase, reprogramming factors) were placed under control of the viral subgenomic promoter which resulted in the high-level production of these proteins in infected cells.

Conclusion: The obtained mutant RNA-virus genome is a promising vector for use in novel systems for epigenetic reprogramming and production of induced pluripotent stem cells.