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Lentiviral vectors as a tool for studying ependyma-specific proteins

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The ependyma is a single-layered, ciliated epithelium at the interface between the parenchyma of the central nervous system and the ventricular space. Recently, it has been implicated in the pathogenesis of hydrocephalus, a debilitating dilation of the cerebral ventricles at the expense of the brain parenchyma. We have previously identified marker proteins for ependymal differentiation and kinocilia generation, namely SCO-C3 and putative adenylate kinase 7 (pAK7). In order to functionally characterize these proteins, it is desirable to introduce corresponding expression and RNAi constructs into our ependymal primary culture model system. However, polyciliated ependymal cells in the primary cultures were found to be completely resistant to transfection with naked plasmid DNA, necessitating more efficient means of nucleic acid transfer. Therefore, a lentiviral vector system based on pseudotyped human immunodeficiency virus (HIV) was established. HIV pseudotyped with the vesicular stomatitis virus envelope protein (VSV-G) and bearing constructs encoding either SCO-C3 or pAK7 were generated and verified for infectivity by successful production of stable HEK293T cell lines expressing the respective proteins. As reported by others, the ependyma is easily transfected by HIV/VSV-G *in vivo*. However, treatment of ependymal primary cultures with this vector resulted in the transfection of only a few polyciliated ependymal cells. While the current transfection efficiency may be insufficient for the proper analysis of target proteins, the principal feasibility of lentiviral transfection of ependymal primary cultures has nevertheless been demonstrated. In order to improve the transfection efficiency, other HIV pseudotypes including HIV/Mokola virus envelope protein, HIV/Rabies virus envelope protein and HIV/Ebola virus envelope protein are being tested. As a byproduct of vector testing, we obtained HEK293T cell lines coexpressing pAK7 fragments and putative pAK7 interaction partners that had previously been identified by yeast two-hybrid screens. These cell lines are now being used to yield additional evidence for the corresponding protein-protein interactions. Cell lines expressing SCO-C3 and pAK7 were further used to test the efficacy of RNAi constructs introduced by other lentiviral vectors. An RNAi sequence yielding almost complete knockdown of pAK7 expression was identified. The successful anti-pAK7 lentivirus is now ready for use in pAK7 knockdown studies both *in vitro* and *in vivo*.