The transduction efficiency of these vectors ranged from 58-97% in CEM cells. After sorting, the transduced cells were all greater than 90% GFP positive. In the more robust HIV infected supernatant re-challenge assay, CEM cells were infected from the primary culture supernatant using equal amounts of p24 antigen. Three lentiviral vector-expressed anti-*Vif* siRNAs (*Vif* A, *Vif* B and *Vif* C) cell lines were active against HIV-1 showing greater than 90% inhibition of viral replication. *Vif* A was the most effective showing greater then 5 logs reduction of viral output. Importantly *Vif* A gave 100% inhibition of HIV genomic RNA and HIV cDNA, confirming the activity of this siRNA towards incoming viral genomic RNA. Conversely *Vif* B and *Vif* C showed no inhibition of HIV RNA and cDNA. In post infection, *Vif* A exhibited a transient reduction of p24 expression while *Vif* B and *Vif* C showed limited effects on HIV p24. Most importantly, all three anti-*Vif* siRNAs yielded defective viral particles resulting in a potent loss of infectivity, presumably a result of loss of Vif and subsequent incorporation of the APOBEC3G cytidine deaminase into the virions and subsequently mutates the viral DNA into non-infectivity.

These results clearly demonstrate that the lentiviral-based vectors can efficiently deliver therapeutic genes into human cells. The anti-*Vif* siRNAs expressed from U6 pol III promoter in lentiviral vectors provide an important therapeutic approach for AIDS gene therapy. First at least one of the anti-*Vif* siRNAs inhibited conversion of viral genomic RNA into DNA, and secondly, all three anti-*Vif* siRNAs reduced *Vif* enough to create defective virions. The *Vif* A siRNA will be recombined with other anti-HIV siRNAs to create a combination of antivirals that should completely prevent the emergence of viral resistance mutations and the combination will be used in primary cells for clinical applications.

9. Coronaviruses: Development of Novel Oncolytic Vectors

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Different viruses are currently being assessed for their capacity to function as anti-tumor agents, supplementing conventional cancer therapies. Here we report on the potentials of a novel oncolytic virus, the mouse hepatitis coronavirus (MHV), in destroying tumor cells *in vitro*. MHV is an enveloped positive strand RNA virus displaying strong species specificity. It has a replication cycle of only 6-9 hours, and rapidly kills murine CEACAM-expressing cells by fusion of the infected cells with their neighboring cells. To redirect MHV to human cancer cells, we produced bispecific adapter molecules binding to the virus as well as to receptors expressed on human cancer cells. The adapters were composed of the ectodomain of the CEACAM receptor coupled to single-chain antibody 425 (directed against the epidermal growth factor receptor), or to a synthetic peptide-tag (directed against an artificial His-tag receptor). Preincubation of the coronaviruses with the adapter proteins and subsequent inoculation of the infection mixes on human cancer cells resulted in receptor specific infection, and cell-cell fusion, typical for coronavirus replication. In order to produce genetically targeted oncolytic coronaviruses, we incorporated the gene encoding the peptide-tag adapter in the MHV genome in a way that during infection continuous amounts of adapter are produced, thereby enabling the progeny virus to reinfect new cells. This resulted in genetically targeted coronaviruses, which were able to specifically infect, and replicate in human cancer cells expressing the specific targeting receptor. We observed multi-round infection, cell-cell fusion, and extensive cell death. These results show that the mouse hepatitis coronavirus, normally not capable of infecting human cells, can be genetically redirected via bispecific adapters to a specific receptor expressed on human cancer cells, consequently leading to rapid cell death. This result provides interesting leads for further investigations on the use of coronaviruses as anti-tumor agents.

10. The Effect of Globin Locus Control Region (LCR) Elements or the MSCV-LTR on Promoter Trapping by Integrated Lentiviral Vector Genomes

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Integrating vectors are desirable for gene therapy of inherited blood disorders to achieve permanent genetic modification and stable expression of transgenes in hematopoietic cells. However, three patients with severe combined immunodeficiency enrolled in a gene therapy trial of a retroviral vector developed leukemia and activation of the *LMO2* proto-oncogene by vector integration has been demonstrated in two. Retroviral vectors prefer the transcriptional start sites for integration while lentiviral vector integration sites are distributed throughout the active genes. Thus, the safety profile of integrating vectors is a critical issue. As a strategy to evaluate interaction of regulatory elements in the vectors with genes into which they have integrated, a series of lentiviral promoter trap vectors were designed to compare the effect of regulatory elements within the vector on the potential for promoter trapping. The trapping cassette consists of a splice acceptor followed by the GFP coding sequences and a polyadenylation site. The cassette was inserted immediately upstream of the 3' LTR in a reverse orientation in vectors containing either a globin LCR element or the oncoretroviral MSCV-LTR also in a reverse orientation to the lentiviral LTR. As a control, a vector containing the cassette without regulatory elements was also constructed. HeLa or erythroid K562 cells were transduced with these vectors and analyzed for GFP expression by flow cytometry. Transduction frequency was estimated by Southern hybridization and the efficiency of promoter trapping was then calculated as a percentage of GFP expressing cells in transduced cells. Our results showed 5% promoter trapping in HeLa cells transduced with the control vector. This efficiency is similar to that recently reported by others (De Palma et al, Blood 2004 Nov 12). Vectors containing either the globin LCR or the MSCV-LTR gave 15 to 20% trapping efficiency. Similarly, in K562 cells, the percentage of promoter trapping of vectors containing either the globin or MSCV regulatory elements was significantly higher (up to 6-fold) of that of the control vector. To further investigate the effect of the globin LCR or the MSCV-LTR on promoter trapping, we isolated single cell clones from GFP-positive HeLa and K562 cells that had been transduced with a trapping vector. Many clones had lost GFP expression when reanalyzed, but the proportion (43%) in which more than 60% of the cells were positive was higher with vectors containing the globin LCR compared to those containing the MSCV-LTR (27%). In addition, the MFI of globin LCR clones was, on average, slightly higher than that of MSCV-LTR clones (20 and 17, respectively). Eighteen clones having a single integrated proviral genome were identified and 15 integration sites have been mapped to date. Ten integration sites were in genes and 8 of these were in the first intron with the promoter trap in tandem with the natural transcript. Our results to date indicate that the presence and nature of regulatory elements within a lentiviral vector influences the probability of vector genome transcription either by trapping of a natural promoter or by activation of cryptic promoters.