viral particles/ml to 10^6 -10^7 viral particles/ml) by ultracentrifugation. Human hematopoietic progenitor cells from cord blood and CD34+ cells isolated from patients with sickle cell (SS) disease were efficiently transduced with this NeoR-containing vector after a single exposure to concentrated RD114 pseudotyped virus produced from the stable RD114 packaging cell line. Up to 78% of progenitors from cord blood and 51% of progenitors from SS CD34+ cells express the NeoR gene as assessed by G418 resistant colonies in methylcellulose. We have also made an RD114 viral producer line with a human β-globin gene-containing retroviral vector construct. Virus from this producer cell line intergrates full length provirus into human Hela target cells and was concentrated to a titer of 5 x 10^6 particles/ml. We used this concentrated RD114 β-globin virus to transfer a human β-globin gene into hematopoietic progenitor colonies from the clinically relevant SS CD34+ cells with our new RD114 stable packaging system. We obtained these SS CD34+ cells from patients with SS disease undergoing exchange transfusion for adverse events in the course of their disease. We find significant numbers of CD34+ cells are spontaneously mobilized in this setting. These data indicate that the RD114 envelope may be useful in stable retroviral and lentiviral packaging systems to permit high-level human β-globin gene transfer and expression for use in human clinical trials in patients with SS disease.

463. Coronaviruses Are Able to Efficiently Eradicate Human Tumor Cells if Provided with the Appropriate Virus Receptor

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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 murine hepatitis coronavirus (MHV), in destroying tumor cells *in vitro*. MHV is a positive strand RNA virus displaying strong species specificity. It has a replication cycle of only 10-15 hours, and efficiently kills cells by fusion of the infected cells with their neighboring cells. Previous studies showed (Kuo L. *et al*., J Virol. 2000;74:1393-406) that the murine host cell tropism of MHV could be changed by substituting its spike protein, necessary for cellular binding and internalization, by the feline coronavirus counterpart. The resulting recombinant coronavirus fMHV only infects feline cells via the feline aminopeptidase N (fAPN) receptor. In this study we showed that the human tumor cell lines HeLa, OVCAR-3, Widr, Caco-2, HCT-81, and HepG2 cells were rendered susceptible to fMHV, as well as to its derivative fMHVdel2aHE, which lacks the non-essential genes 2a and HE, by transfection with a fAPN expression construct. Furthermore, fMHV and fMHVdel2aHE infections of HeLa and OVCAR-3 cells stably expressing fAPN, resulted in rapid killing of both cell cultures starting at 12-24 h post-inoculation. In addition, 3-D multilayer OVCAR-fAPN spheroids established from 5 x 10^4 cells could be eradicated within 7 days of inoculation with 5 x 10^4 PFU fMHV, which was not accomplished by inoculating 5 x 10^8 IU wild-type adenovirus, a DNA virus used frequently in studies on tumor eradication. The deletion mutant fMHVdel2aHE was attenuated as compared to fMHV, although it was still as efficient in destroying OVCAR-fAPN spheroids as wild-type adenovirus using these dosages. These results show that infections with coronaviruses, if retargeted to human tumor cells, cause rapid and efficient death of these cells. Hence, tumor-targeted coronaviruses seem interesting as anti-tumor agents.

464. Engineering the Splice Acceptor for Improved Gene Expression and Viral Titer in an MLV-Based Retroviral Vector

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We have recently developed a series of retroviral vectors that are absent of any viral coding sequences yet still drive significantly higher levels of gene expression than other well known MLV-based vectors. This has been accomplished by introducing heterologous intron and exon sequences, harboring the spice acceptor from the human EF-1α gene and the major IE region of human cytomegalovirus. It was found that in these vectors, the subgenomic RNA containing the target gene was produced at a high level. However, one downside of using the efficient splice acceptor sequence was that viral titer significantly varied depending on the packaging lines used. For example, viral titer was comparable between the vectors containing and lacking the splice acceptor sequence in Phoenix, but differed by 3 to 10 fold in FLYA13 and PG13. The Northern blot analysis indicated that in the latter packaging lines, the genomic transcript containing the packaging signal sequence was efficiently spliced to the subgenomic RNA, resulting in low levels of the genomic RNA and thus leading to low viral titer. We tested the possibility of overcoming this problem by manipulating the splice acceptor in such a way that a delicate balance was maintained between the splicing efficiency (which determines the level of gene expression) and the amount of the genomic transcript (which influences viral titer). After extensive mutational analysis on the splice acceptor from the EF-1α gene as a model, several mutations were found to fulfill such a requirement. One of the newly developed vectors called MT5 can drive higher levels of gene expression than other control vectors without compromising viral titer. This type of vector should be useful in actual clinical settings because; (1) it contains no viral coding sequence, thus minimizing any homologous recombination between the vector and the packaging genome; and, (2) it drives high levels of gene expression, while maintaining viral titer comparable to other known vectors.

465. Promoter Trap Vectors for Transcriptional Targeting: A Combinatorial Approach

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Transcriptional-targeting, defined as the use of tissue-specific enhancer/promoter elements, is an important goal of gene therapy. Unfortunately, the majority of promoters used in gene therapy are either viral in origin, or function sub-optimally in the absence of a restricting domain structure that may be far larger than the vector permits. Mouse virus-like 30S (VL30) elements are expressed in most tissues and have tissue-specific long terminal repeats (LTRs), making it possible to rescue the relatively short, tissue-specific promoters from RNA or cDNAs from the tissues or cells of choice. The VL30 vector system exactly replaces the murine leukemia virus system. We constructed promoter trapping vectors (Fig.1), consisting of VL30 *cis*-acting sequences, having a deletion in the 3'-LTR. A technique was devised whereby the promoters can be amplified from RNA or DNA and inserted directly into the LTR, without disturbing highly-conserved LTR boundary domains. After the vector is expressed in retrovirus producer cells, the tissue-specific promoter is transferred to the 5'-LTR during replication, permitting infection of the target cells with a retro-vector expressing the foreign genes via the tissue-specific promoter. A combinatorial promoter

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