Either of these approaches may facilitate the preparation of lentiviral vectors for large-scale preclinical studies and, ultimately, for clinical applications in humans.

454. Incorporation of Modified Vesicular Stomatitis Virus G-Glycoprotein (VSV-G) into Infectious Pseudotyped Retrovirus Vectors

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Studies of cell-targeted gene delivery by VSV-G-pseudotyped retroviruses have been hampered partly by the lack of three-dimensional structural characterization of the VSV-G protein. Modified variants of VSV-G containing potentially targeting ligands have been found to interfere with vector production by disruption of normal intracellular trafficking mechanisms or defective fusiogenic properties of the modified VSV-G protein, with resulting ineffective assembly into infectious virus particles. In the current study, we demonstrate production of infectious virus from retrovirus packaging cells expressing VSV-G proteins modified to contain potentially targeting ligands introduced into a site of VSV-G identified from primary structural predictions. The inserted ligands include an octapeptide previously identified by phage display methods to home to myocardial endothelium or the collagen binding domain of Von Willebrand factor previously reported to target non-pseudotyped retroviruses to collagen-displaying tissues in vivo. Under standard tissue culture virus production conditions at the restrictive temperature of 37°C, deconvolution microscopy reveals that both modified VSV-G molecules accumulate in peri-nuclear structures and fail to translocate effectively to the cell membrane, thereby becoming unavailable for assembly of infectious virus particles. However, at the lower permissive temperature of 30°C, modified VSV-G proteins traffic appropriately to the cell surface and produce infectious virus at titers of approximately 10e4 infectious units/ml. Studies of the potential targeting properties of these vectors are underway.

455. Targeting of a Non-Human Coronavirus to Human Tumor Cells by Using a Bispecific Single-Chain Antibody

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Currently, several viruses are being assessed for their use as anti-tumor agents. For this purpose, targeting of viruses to epitopes on tumor cells is often essential to achieve a tumor-specific infection. In this study, we determined whether a recombinant non-human coronavirus, i.e. a mouse hepatitis virus with feline tropism (fMHV), could be targeted to an epitope over-expressed on human tumor cells. The main advantages of coronaviruses over the currently used viral vectors when aiming at viral tumor therapy are that they have a short replication cycle and, consequently, lead to rapid killing of their target cells. Here, we constructed a recombinant bispecific antibody, consisting of a neutralizing anti-feline spike protein single chain variable fragment (scFv) antibody (23F) fused to a scFv (425) directed against the epidermal growth factor receptor (EGFR). The resulting bispecific scFv protein mediated fMHV infection on a panel of EGFR-expressing human cancer cell lines, including HeLa, OVCAR-3, HCT-81, Widr, Caco-2, and HepG2 cells, as demonstrated by immunostaining with a virus-specific MAb. In contrast, inoculation of these cell lines with fMHV in the absence of bispecific scFv protein did not result in any detectable infection. In addition, a derivative of the murine cell line NIH-3T3 stably expressing the EGFR could be infected with fMHV, whereas parental NIH-3T3 cells could not, confirming the specificity of the infection via EGFR. This was further corroborated by the finding that infection of human tumor cell lines using the bispecific fusion protein could be blocked with MAb 23F8.1 or Mab 425, the anti-spike and anti-EGFR antibodies respectively, from which the two scFv were derived. In conclusion, fMHV can be selectively targeted to human tumor cells by using a bispecific targeting device. This result provides a rationale for further investigations on the use of coronaviruses as anti-tumor agents.

456. Development of a High-Titer B Cell-Specific Self-Inactivating Retroviral Vector

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Gene therapy has been impeded by the lack of cell-specific vectors that direct gene expression to an appropriate progeny of transduced pluripotent hematopoietic stem cells (HSC). The use of self-inactivating retroviral vectors (SIN) with a deletion of the viral enhancer/promotor and an internal eukaryotic promotor driving cell-specific transgene expression is complicated by very low viral titers. To address the influence of the posttranscriptional regulatory element of the woodchuck hepatitis virus (WPRE) on viral titers and gene expression in multiple lineages of the hematopoietic system *in vivo*, we used a SIN-vector containing the promoter/enhancer of the Spleen Focus Forming Virus (SFFV) as internal promoter and eGFP as marker, with and without the addition of WPRE. We could show that WPRE increases the titer of SIN-vectors up to 15-fold. Under conditions of identical MOI (multiplicity of infection), enhancement of gene expression was observed *in vitro* in NIH3T3 cells and primary bone marrow cells. The transduced bone marrow cells were subsequently used to generate bone marrow chimeric mice, but in contrast to the *in vitro* data, no benefitial effect on gene expression could be observed in different hematopoietic lineages *in vivo*. With the high-titer WPRE-SIN vector, 75% to 87% of B-cells, 87% to 92% of CD11b cells and 61% to 81% of T cells were transgenic (eGFP+). In contrast, only 19% to 24% of B cells, 35% to 53% of CD11b cells and 15% to 23% of T cells expressed eGFP when the SIN-vector lacking WPRE was employed. Our data show that the use of WPRE in combination with SIN-vectors enhances their viral titers and therefore the number of transduced target cells, but not gene expression itself *in vivo*.

In order to develop a retroviral SIN-vector which could be useful in hematopoietic stem cell gene therapy for B cell lineage disorders, we replaced the SFFV promoter/enhancer in the WPRE-containing vector with the human CD19 promoter, and bone marrow chimeric mice were generated. We could detect cell-specific gene expression in 15% to 35% of B cells in the blood. In addition, long-term *in vivo* expression in all B cell developmental stages was observed in blood, bone marrow, and secondary lymphatic organs.