

Retrovirus Vectors Bearing Jaagsiekte Sheep Retrovirus Env Transduce Human Cells by Using a New Receptor Localized to Chromosome 3p21.3

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Jaagsiekte sheep retrovirus (JSRV) is a type D retrovirus associated with a contagious lung tumor of sheep, ovine pulmonary carcinoma. Other than sheep, JSRV is known to infect goats, but there is no evidence of human infection. Until now it has not been possible to study the host range for JSRV because of the inability to grow this virus in culture. Here we show that the JSRV envelope protein (Env) can be used to pseudotype Moloney murine leukemia virus (MoMLV)-based retrovirus vectors and that such vectors can transduce human cells in culture. We constructed hybrid retrovirus packaging cells that express the JSRV Env and the MoMLV Gag-Pol proteins and can produce JSRV-pseudotype vectors at titers of up to 10⁶ alkaline phosphatase-positive focus-forming units/ml. Using this high-titer virus, we have studied the host range for JSRV, which includes sheep, human, monkey, bovine, dog, and rabbit cells but not mouse, rat, or hamster cells. Considering the inability of the JSRV-pseudotype vector to transduce hamster cells, we used the hamster cell line-based Stanford G3 panel of whole human genome radiation hybrids to phenotypically map the JSRV receptor (JVR) gene within the p21.3 region of human chromosome 3. JVR is likely a new retrovirus receptor, as none of the previously identified retrovirus receptors localizes to the same position. Several chemokine receptors that have been shown to serve as coreceptors for lentivirus infection are clustered in the same region of chromosome 3; however, careful examination shows that the JSRV receptor does not colocalize with any of these genes.

Jaagsiekte sheep retrovirus (JSRV) is the causative agent of a contagious lung cancer of sheep known as ovine pulmonary carcinoma (OPC), also known as sheep pulmonary adenomatosis or jaagsiekte. OPC is a veterinary problem with significant economic impact in several countries. In addition, OPC shares characteristics with human bronchioalveolar carcinoma (BAC) (12, 32, 49), and BAC represents about 25% of human lung cancer cases (6). Lung cancer being the most common fatal form of cancer in humans (10), recent interest in JSRV stems from the hypothesis that OPC could be useful as a naturally occurring animal model for understanding the mechanism of pulmonary carcinogenesis (26, 49).

JSRV has been classified as a type D retrovirus, based on genomic organization, but has a type B-like Env protein (61). The sheep genome carries multiple copies of JSRV-like endogenous sheep retrovirus (ESRV) sequences (25, 27, 61), but subsequent studies have shown that JSRV is an exogenous virus distinct from ESRV sequences (4, 5, 44) and is specifically associated with OPC. Recent studies by Palmarini et al. (48) using an infectious molecular clone of JSRV have confirmed that JSRV is the causative agent of OPC. The mechanism of oncogenesis by JSRV is not known. JSRV has the genomic organization of a simple replication-competent retrovirus with no known oncogenes. The incubation period in naturally acquired OPC seems to range from months to years, suggesting

insertional mutagenesis. However, OPC can be induced experimentally in 3 to 4 weeks, suggesting a mechanism of action more similar to that of a transforming retrovirus.

The main sites of JSRV replication and assembly are the transformed epithelial cells of the lung, especially the alveolar type II cells (45). The lung fluid and tumor extracts of infected sheep can be used for virus isolation and also for experimentally transmitting the disease to lambs by intratracheal inoculation (14, 37, 59), suggesting that the virus is stable in lung fluid. The stability of the virus in lung fluid as well as the ability to infect the epithelial cells of the lung indicate that JSRV vectors may be useful for gene transfer into the lung for human diseases such as cystic fibrosis. Even if the vectors were unable to transduce human airway epithelial cells, characterization of this virus would provide insights into developing improved vectors for transduction of the lung.

To study JSRV as a prospective retroviral vector, we first wanted to test whether the JSRV Env protein could be used to pseudotype a commonly used Moloney murine leukemia virus (MoMLV)-based retrovirus vector. Once this was established by transient transfection and the JSRV Env was shown to promote entry into human cells, we generated a packaging cell line for producing high-titer JSRV-pseudotype retrovirus vector which allowed us to investigate the host range for JSRV Env. Study of the JSRV host range has been hindered so far by the lack of an in vitro culture system for growing the virus, and only recently has an infectious molecular clone of the virus been developed (48). Subsequently, we used a human-hamster whole genome radiation hybrid (RH) panel to precisely localize the JSRV receptor (JVR) within human chromosome 3p21.3.

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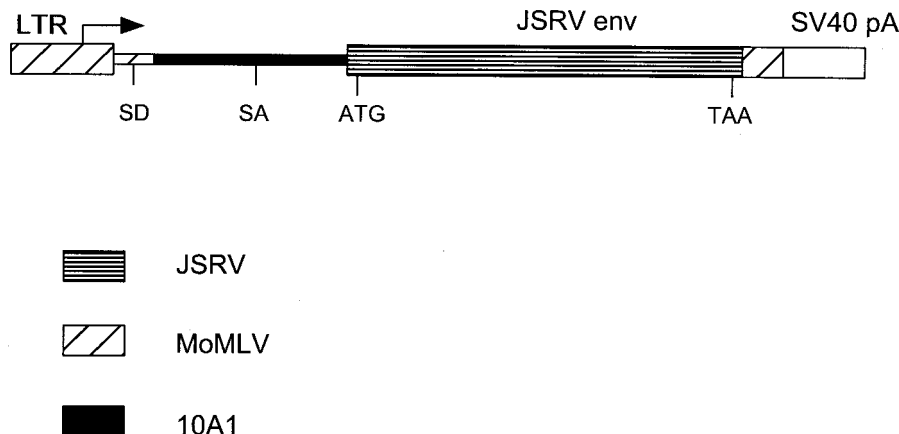


FIG. 1. JSRV Env expression plasmid pSX2.Jenv. Abbreviations: SV40 pA, the early-region polyadenylation signal from simian virus 40; LTR, retrovirus long terminal repeat; SD and SA, splice donor and acceptor sites, respectively. The ATG start and TAA stop codons of Env are shown.

MATERIALS AND METHODS

Cell culture. Mammalian cells, including SSF-123 primary sheep skin fibroblasts (gift from William Osborne, University of Washington, Seattle), HT-1080 human fibrosarcoma cells (American Type Culture Collection [ATCC] cell line CCL-121), 293 human kidney epithelial cells (ATCC CRL 1573), IB3 immortalized human bronchial epithelial cells (62), HeLa cervical carcinoma cells (ATCC CCL-2), NIH 3T3 thymidine kinase-deficient mouse embryo fibroblasts (60), *Mus dunni* tail fibroblasts (11), D17 canine osteosarcoma cells (ATCC CRL-6248), 208F rat embryo fibroblasts (51), MDBK bovine kidney epithelial cells (ATCC CCL-22), Vero African green monkey kidney epithelial cells (ATCC CCL-81), MF-NAN primary mouse (BALB/c) fibroblasts, MF-H1 primary mouse (C57BL/6) fibroblasts, and RbTE rabbit tracheal epithelial cells (gifts from Christine Halbert, Fred Hutchinson Cancer Research Center), were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hyclone). RbTE cells were immortalized by transduction with the human papillomavirus E6 and E7 genes in a retrovirus vector, LXSN16E6E7 (24). G355 feline embryonic brain cells (15) were grown in McCoy's medium supplemented with 15% fetal bovine serum. CHO cells (ATCC CCL-61), A23 hamster cells, and the A23-derived RH clones (57) (gifts from Davis Cox, Stanford University) were grown in minimal essential medium- α supplemented with 10% fetal bovine serum.

Retroviral vectors and virus titer. The nomenclature for retroviral vectors and pseudotypes has been discussed before (41). LAPSIN is an MoMLV-based vector encoding the human placental alkaline phosphatase (AP) and the neomycin phosphotransferase proteins (42). Vectors with a JSRV pseudotype were made by using the pSX2.Jenv plasmid (Fig. 1), which was constructed by inserting the 1,883-bp *MslI-Ecl136* fragment of JSRV_{-JS7} containing the Env coding region into the *BsaAI*- and *MscI*-cut 4,239-bp backbone of the pSX2 plasmid (38) by blunt-end ligation. JSRV_{-JS7} is a proviral clone derived from a λ phage library of genomic DNA from the JS7 cell line that was derived from a spontaneous case of OPC (unpublished results). Retrovirus vector titers were determined as described previously, by assaying for either AP⁺ focus-forming units (FFU) (17) or G418-resistant colony-forming units (CFU) (40).

Generation of JSRV-pseudotype retrovirus packaging cells. Stable retrovirus packaging cells expressing the JSRV Env were made using the techniques described previously for the construction of 10A1 murine leukemia virus (MLV)-pseudotype packaging cell lines (38). Briefly, NIH 3T3 cells that express the MoMLV Gag-Pol proteins (LGPS cells [39]) were cotransfected with plasmid pSX2.Jenv and a plasmid encoding hygromycin phosphotransferase (pSV2 Δ 13-hyg; gift from Paul Berg, Stanford University) at a 20:1 or 100:1 ratio, and 24 hygromycin-resistant clones were isolated. These clonal cell lines were tested for their ability to produce retrovirus vectors by measuring the titer of vector produced by the cells after the cells were transduced with the LAPSIN vector made by using PT67 packaging cells (38). HT-1080 human cells were used as targets for measurement of the vector titer. The clonal line that produced the highest-titer vector was clone PJ14.

Marker rescue assay. SSF and HT-1080 cells were plated at 10^5 cells per 6-cm dish on day 1, transduced with LAPSIN(PT67) virus at a multiplicity of infection of ~ 1 in the presence of Polybrene (4 μ g/ml) on day 2, and trypsinized and replated in G418 (active concentration of 250 μ g/ml for SSF and 400 μ g/ml for HT-1080 cells). These polyclonal populations of cells carrying the LAPSIN vector were used in the marker rescue assay for helper virus as described before (38). Briefly, SSF/LAPSIN or HT-1080/LAPSIN cells were plated at 5×10^5 cells per 6-cm dish on day 1, infected with 0.5 ml of LAPSIN(PJ) test virus (6×10^5 AP FFU/ml) per dish in the presence of Polybrene on day 2, and trypsinized and split

1:10 every 2 to 3 days for 2 weeks while being kept at high density to facilitate potential virus spread. After 2 weeks, medium harvested from confluent dishes of cells was tested for LAPSIN vector rescue and transfer using SSF cells as targets. MoMLV was used as a positive control to show that helper virus could rescue the LAPSIN vector in these cells. The passaged SSF/LAPSIN and HT-1080/LAPSIN cells were also stained for AP to ensure that they retained the LAPSIN vector.

RH mapping of JVR and STRL33 genes. The Stanford G3 panel of human whole-genome hybrid cell lines was used for phenotypic RH mapping (www-shgc.stanford.edu/Mapping/rh/) (57). For mapping JVR, the RH cell lines were plated at 5×10^4 cells per well in a 6-well plate and exposed to LAPSIN(JSRV) vector the next day. AP assays were performed as explained above, and AP⁺ FFU were counted to measure transduction.

To map the STRL33 gene, we used the Stanford G3 panel RH DNA (Research Genetics, Huntsville, Ala.) for genotypic mapping. PCR was performed with the STRL33-specific primers 5'-GCCAGGGTTTCGAGAAGCTGCTCTG GAATT-3' and 5'-TCATAGTCCCTGGTGCTAGTTATTCTGGAT-3'. Genomic DNA samples from the A3 hamster cell line (57) and the RM human lymphoblastoid cell line (57) were used as negative and positive controls, respectively. All PCR amplifications were performed with an initial denaturation step at 94°C for 2 min, followed by 35 cycles of amplification at 94°C for 30 s, at 62°C for 1 min, and 68°C for 4 min, with a final extension at 68°C for 10 min. The PCR products were electrophoresed on 1% agarose gels and visualized by ethidium bromide staining.

RESULTS

JSRV Env protein can pseudotype an MoMLV-based retrovirus vector and promote transduction of human cells. We tested whether the JSRV Env could be incorporated into virions containing MoMLV Gag-Pol proteins and an MoMLV-based vector by transient transfection of the JSRV Env expression plasmid pSX2.Jenv (Fig. 1) into LGPS/LAPSIN cells. These cells contain the MoMLV-based LAPSIN vector and the pLGPS plasmid for expression of MoMLV Gag-Pol proteins (38). Transfection of the pSX2 plasmid DNA expressing the 10A1 MLV Env (38) was used as a positive control. Two days after transfection, the medium from these cells was harvested and assayed for vector production using SSF-123 sheep skin fibroblasts and HT-1080 human fibrosarcoma cells as targets for transduction (Table 1). Both cell types could be transduced by the JSRV-pseudotype LAPSIN vector, showing the ability of JSRV Env to pseudotype MoMLV-based vectors packaged with MoMLV Gag proteins as well as the ability of JSRV Env to promote entry into human cells. The JSRV-pseudotype vector titer was ~ 10 -fold higher on the sheep than on the human cells. In contrast, the titers of the 10A1- and xenotropic-pseudotype LAPSIN vectors were at least 10-fold higher on the human than on the sheep cells, showing the preference of the JSRV-pseudotype vector for sheep cells. These results indi-

TABLE 3. Chromosomal localization of retrovirus receptors in the human genome

Retrovirus group	Receptor name(s)	Localization	Method ^a	Reference(s) ^b
Ecotropic MLV	CAT1 (Rec1, SLC7A1)	13q12	In situ hybridization, RFLP	1
GALV/feline leukemia virus type B	PIT1 (GLVR1, SLC20A1)	2q11-q14	In situ hybridization	33
Amphotropic MLV	PIT2 (Ram1, GLVR2, SLC20A2)	8p11-q11	Somatic cell hybrids	19
Xenotropic and polytropic MLV	XPR1	1q25.1	RH	8
RD114/simian type D retrovirus	RDR (SLC1A5)	19q13.3	RH	52
Feline leukemia virus type C	FLVCR	1q32.1	RH	A
T-cell leukemia viruses (human and simian T-cell lymphotropic viruses)	HTLVR	17q23	Somatic cell hybrids	20, 58
Lentiviruses (human, simian, and feline immunodeficiency viruses)	CD4	12p12-p13	DNA sequencing	3
	CCR1, -2b, -3, -5	3p21.3	DNA sequencing, RH	55
	CCR4	3p22 ^c	RH	55
	CCR8	3p22	RH	56
	CXCR4	2q21	In situ hybridization	16
	CX3CR1 (V28)	3p22	FISH, RH	13, 35
	GPR1	15q26.1	FISH	36
	GPR15 (Bob)	3q11.2-q13.1	FISH	29
	ChemR23	12q21.2-q21.3	RH	54
	APJ	11q12	FISH	43
	STRL33 (Bonzo, TYMSTR)	3p21.3	RH, somatic cell hybrids	34, B
	JSRV	JVR	3p21.3	RH

^a RFLP, restriction fragment length polymorphism; FISH, fluorescent in situ hybridization.

^b A, J.-L. Battini, J. E. J. Rasko, and A. D. Miller, unpublished data; B, this report.

^c The localization of CCR4 given by the authors (3p24) was changed to 3p22 to reflect more recent localization data for flanking markers.

localized (34). These studies indicated that JVR is probably a new retroviral receptor but might be the same as STRL33.

STRL33 (Bonzo) maps to 3p21.3 region, ~500 kb telomeric to the 285-kb CCR cluster. To determine whether or not STRL33 might function as the JSRV receptor, we used the Stanford G3 panel RH DNA for mapping the STRL33 gene, as explained in Materials and Methods. The PCR results for DNA samples from the 83 ordered hybrids was 0000000000000100001100000000000000000000010100R001000000000000000010000110000000000000000000001010010000R001000010, where 0 indicates PCR negative, 1 indicates PCR positive, and R indicates an indeterminate result. This bar code was submitted to the SHGC RH Server v4.0, which mapped STRL33 at a distance of 13 cR_{10,000} (LOD, 9.46) from marker SHGC-12886. Using the multiple integrated maps at the NCBI Entrez Genomes site, we have localized the STRL33 gene to about 500 kb away from the CCR cluster in chromosome 3p21.3 and about 7.5 Mb from JVR, thus showing that STRL33 and JVR do not localize to the same position and JVR is not likely to be any of the known retroviral receptors (Fig. 2).

DISCUSSION

JSRV has recently gained prominence mainly because of the similarity of OPC to BAC in humans, suggesting that OPC can be used as an animal model to understand the process of pulmonary carcinogenesis. However, studies on JSRV have been hindered so far by the lack of a cell culture system for propagating the virus. Recently, a full-length infectious proviral molecular clone of JSRV was isolated from a natural case of OPC, and a cell culture system was developed to propagate

the virus by replacing the upstream U3 with the cytomegalovirus early promoter (48). JSRV has been known to infect sheep and goats, but there is no evidence of human infection. Our studies show that the JSRV Env can be used to pseudotype MoMLV-based retroviral vectors containing MoMLV Gag-Pol proteins, thus providing a means for studying the host range of the viral Env protein. We have been able to generate packaging cell lines based on JSRV Env and MoMLV Gag-Pol proteins that can produce JSRV-pseudotype retroviral vectors at titers of up to 10⁶ AP⁺ FFU/ml. Our results show that the JSRV Env promotes entry into sheep, human, monkey, bovine, dog, and rabbit cells but not mouse (laboratory or wild), rat, or hamster cells. The inability of the vector to transduce mouse or rat cells is unfortunate, as it prevents us from using rodents for studying JSRV pathogenesis or in vivo gene transfer to the lung. Poor transduction of HeLa cells suggests that the JSRV receptor (JVR) may not be constitutively expressed in all cell types.

This is the first report showing the ability of JSRV Env to transduce human cells and suggesting the feasibility of developing JSRV as a prospective retroviral vector for gene transfer to the lung. Although both viral (18, 53) and nonviral (2, 31) methods have been extensively studied, efficient gene transfer to the airway epithelial cells has proved difficult (21, 22, 50). Previous studies in our laboratory have shown that amphotropic retroviral vectors can efficiently transduce the basal and secretory airway epithelial cells in vitro, but in vivo delivery resulted in no detectable transduction in the intact normal airway epithelium and a low transduction rate in the wounded epithelium (23). This low retroviral transduction in vivo is due

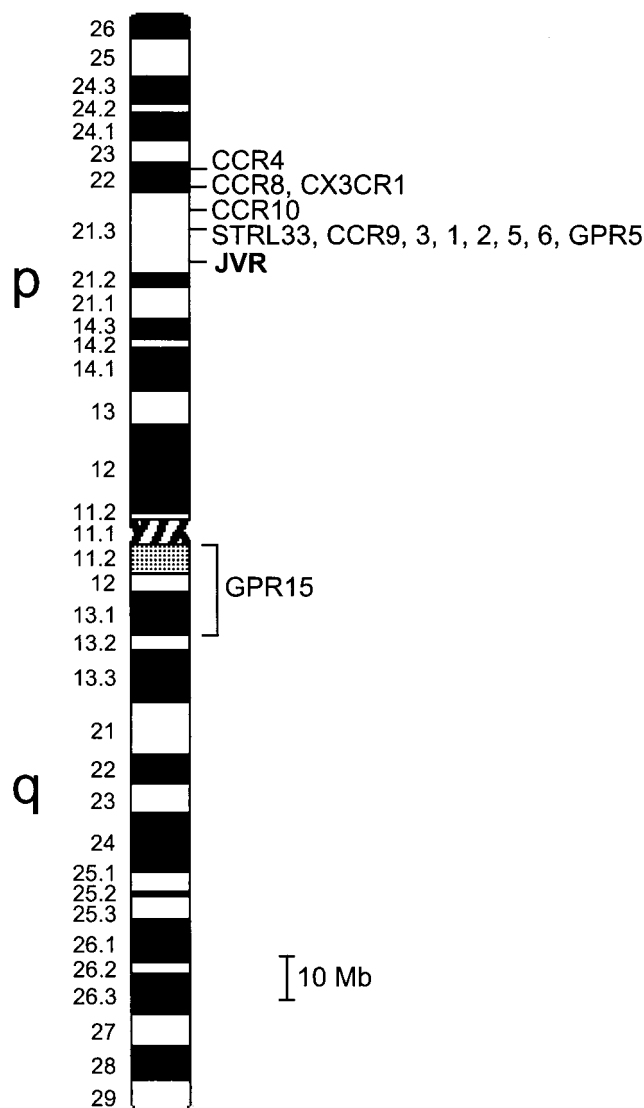


FIG. 2. Retrovirus receptor and related G protein-coupled receptor genes on chromosome 3. Human chromosome 3 is ~240 Mb in length, and an idiogram of this chromosome at the 550-band level is shown. Rough localizations are shown by brackets, while more precise localizations are shown by lines. Where the genes are too closely spaced to show the order on a map of this resolution, the genes are listed in order from telomere to centromere. References for the retrovirus receptor localizations are given in Table 3, and those for the related proteins are CCR9 (35), CCR10 (9, 35), and GPR5 (28). Relationships between distances (in centirays), determined by RH analysis and physical genome length, were derived primarily by using data from the multiple integrated maps of the WWW Entrez Genomes Division of the NCBI.

to the low abundance of retroviral receptors and inhibition of amphotropic retroviral vector transduction by pulmonary surfactant (63) or by soluble chondroitin sulfates in pleural effusions (7). Although JSRV infects several cell types in vivo (30, 45, 47, 48), the epithelial tumor cells in the lungs of sheep have been shown to be the major sites of viral replication (45), suggesting a natural tropism of the virus for the airway epithelial cells. Furthermore, OPC can be experimentally reproduced in newborn lambs by intratracheal inoculation of concentrated lung fluid or tumor extracts collected from OPC-affected sheep (14, 37, 59), demonstrating the stability of the virus in lung fluid. We are currently studying the ability of JSRV to infect

airway epithelial cells and its stability in the presence of pulmonary surfactant.

The ability of JSRV Env to promote infection of human cells in culture could be relevant to the epidemiology of human lung cancer, especially with regard to nonsmokers exposed to sheep in which OPC is endemic. Although there is no proof for JSRV involvement in human lung carcinoma, the possibility of viral etiology cannot be excluded because of the similarity of BAC to OPC and the multifocal and multiclonal nature of some BAC cases (46). Several factors could explain the absence of evidence for human infection with JSRV, such as lack of immunological reagents to detect human infections. There has been no report of any serological study to evaluate human sera for JSRV antibodies. Alternatively, the JSRV Env might be able to bind to receptors and mediate entry of the viral genome, but some of the viral replicative elements may not be functional in human cells, resulting in postentry or replication blocks. It is known that the JSRV Gag-Pol proteins are functional during viral assembly in human cells, as evidenced by the use of an infectious molecular clone of JSRV to produce the virus in 293 human epithelial cells (48). However, their functionality during reverse transcription and integration of viral DNA in human cells is unknown. Another important factor might be the presence of transcriptionally active ESRV sequences in the sheep genome, which may induce tolerance to JSRV antigens in sheep and allow the virus to propagate and establish an infection. On the contrary, humans and other animals may develop a strong immune response leading to virus clearance.

Chromosome localization provides an important alternative approach to interference analysis to determine retroviral receptor usage. The advantages of chromosome localization are that extensive cross-interference analyses between the test virus and the growing number of existing viruses need not be performed, and the technique is informative for viruses that do not exhibit strong interference to infection by viruses that use the same receptor. Utilizing the inability of JSRV Env to promote infection of hamster cells, we have used a panel of human-hamster whole-genome RH cell lines to localize the JSRV receptor (JVR) gene to the p21.3 region of human chromosome 3. Although the majority of known retroviral receptors do not localize to chromosome 3, most of the CC-chemokine receptor genes (CCRs) which have been identified as coreceptors for lentiviruses have been shown to map within the 3p21.3 region (55). Careful analysis of the mapping data revealed that JVR does not map to the same positions as most of these receptors, being ~7 Mb away from the 285-kb cluster of CCR3, -1, -2, -5, and -6 and farther away from CCR4, CCR8, and CCR10 (Fig. 2). The lentivirus receptor CX3CR1 has recently been mapped to the 3p24 region (35), leaving one other lentivirus receptor, STRL33 (Bonzo), in question (34). Using the G3 panel of RH DNA, we have localized the STRL33 gene 500 kb telomeric to the CCR cluster in region 3p21.3 and about 7.5 Mb away from JVR. These results indicate that JVR is a new retroviral receptor in human cells.

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REFERENCES

1. Albritton, L. M., A. M. Bowcock, R. L. Eddy, C. C. Morton, L. Tseng, L. A. Farrer, L. L. Cavalli-Sforza, T. B. Shows, and J. M. Cunningham. 1992. The human cationic amino acid transporter (ATRC1): physical and genetic mapping to 13q12-q14. *Genomics* **12**:430-434.
2. Alton, E., P. Middleton, N. Caplen, S. Smith, D. Steele, F. Munkonge, P. Jeffrey, D. Cieddes, S. Hart, R. Williamson, K. Fasold, A. Miller, P. Dickenson, B. Stevenson, G. McLachlan, J. Dorin, and D. Porteous. 1993. Non-invasive liposome-mediated gene delivery can correct the ion transport defect in cystic fibrosis mutant mice. *Nat. Genet.* **5**:135-142.
3. Ansari-Lari, M. A., D. M. Muzny, J. Lu, F. Lu, C. E. Lilley, S. Spanos, T. Malley, and R. A. Gibbs. 1996. A gene-rich cluster between the CD4 and triosephosphate isomerase genes at human chromosome 12p13. *Genome Res.* **6**:314-326.
4. Bai, J., J. V. Bishop, J. O. Carlson, and J. C. DeMartini. 1999. Sequence comparison of JSRV with endogenous proviruses: envelope genotypes and a novel ORF with similarity to a G-protein-coupled receptor. *Virology* **258**:333-343.
5. Bai, J., R.-Y. Zhu, K. Stedman, C. Cousens, J. Carlson, J. M. Sharp, and J. C. DeMartini. 1996. Unique long terminal repeat U3 sequences distinguish exogenous jaagsiekte sheep retrovirus associated with ovine pulmonary carcinoma from endogenous loci in the sheep genome. *J. Virol.* **70**:3159-3168.
6. Barsky, S. H., R. Cameron, K. E. Osann, D. Tomita, and E. C. Holmes. 1994. Rising incidence of bronchioalveolar carcinoma and its unique clinicopathologic features. *Cancer* **73**:1163-1170.
7. Batra, R. K., J. C. Olsen, D. K. Hoganson, B. Caterson, and R. C. Boucher. 1997. Retroviral gene transfer is inhibited by chondroitin sulfate proteoglycans/glycosaminoglycans in malignant pleural effusions. *J. Biol. Chem.* **272**:11736-11743.
8. Battini, J.-L., J. E. J. Rasko, and A. D. Miller. 1999. A human cell-surface receptor for xenotropic and polytropic murine leukemia viruses: possible role in G protein-coupled signal transduction. *Proc. Natl. Acad. Sci. USA* **96**:1385-1390.
9. Bonini, J. A., S. K. Martin, F. Dralyuk, M. W. Roe, L. H. Philipson, and D. F. Steiner. 1997. Cloning, expression, and chromosomal mapping of a novel human CC-chemokine receptor (CCR10) that displays high-affinity binding for MCP-1 and MCP-3. *DNA Cell Biol.* **16**:1249-1256.
10. Carney, D. N., and L. De Leij. 1988. Lung cancer biology. *Semin. Oncol.* **15**:199-214.
11. Chattopadhyay, S. K., M. R. Lander, S. Gupta, E. Rands, and D. R. Lowy. 1981. Origin of mink cytopathic focus-forming (MCF) viruses: comparison with ecotropic and xenotropic murine leukemia virus genomes. *Virology* **113**:465-483.
12. Clayton, F. 1988. The spectrum and significance of bronchioalveolar carcinomas. *Pathol. Annu.* **23**:361-394.
13. Combadiere, C., S. K. Ahuja, and P. M. Murphy. 1995. Cloning, chromosomal localization, and RNA expression of a human beta chemokine receptor-like gene. *DNA Cell Biol.* **14**:673-680.
14. DeMartini, J. C., R. H. Rosadio, J. M. Sharp, H. I. Russell, and M. D. Lairmore. 1987. Experimental coinfection of type D retrovirus-associated pulmonary carcinoma and lentivirus-associated lymphoid interstitial pneumonia in lambs. *J. Natl. Cancer Inst.* **79**:167-177.
15. Dunn, K. J., C. C. Yuan, and D. G. Blair. 1993. A phenotypic host range alteration determines RD114 virus restriction in feline embryonic cells. *J. Virol.* **67**:4704-4711.
16. Federspiel, B., I. G. Melhado, A. M. V. Duncan, A. Delaney, K. Schappert, I. Clark-Lewis, and F. R. Jirik. 1993. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. *Genomics* **16**:707-712.
17. Fields-Berry, S. C., A. L. Halliday, and C. L. Cepko. 1992. A recombinant retrovirus encoding alkaline phosphatase confirms clonal boundary assignment in lineage analysis of murine retina. *Proc. Natl. Acad. Sci. USA* **89**:693-697.
18. Flotte, T. R., S. A. Affione, C. Conrad, S. A. McGrath, R. Solow, H. Oka, P. L. Zietlin, W. B. Guggino, and B. J. Carter. 1993. Stable *in vivo* expression of the cystic fibrosis transmembrane regulator with adeno-associated virus vector. *Proc. Natl. Acad. Sci. USA* **90**:10613-10617.
19. Garcia, J. V., C. Jones, and A. D. Miller. 1991. Localization of the amphotropic murine leukemia virus receptor gene to the pericentromeric region of human chromosome 8. *J. Virol.* **65**:6316-6319.
20. Gavalchin, J., N. Fan, P. G. Waterbury, E. Corbett, B. D. Faldaz, S. M. Peshick, B. J. Poesz, L. Papsidero, and M. J. Lane. 1995. Regional localization of the putative cell surface receptor for HTLV-I to human chromosome 17q23.2-17q25.3. *Virology* **212**:196-203.
21. Goldman, M. J., P. S. Lee, J. S. Yang, and J. M. Wilson. 1997. Lentiviral vectors for gene therapy of cystic fibrosis. *Hum. Gene Ther.* **8**:2261-2268.
22. Grubb, B. R., R. J. Pickles, H. Ye, J. R. Yankaskas, R. N. Vick, J. F. Engelhardt, J. M. Wilson, L. G. Johnson, and R. C. Boucher. 1994. Inefficient gene transfer by adenovirus vector to cystic fibrosis airway epithelia of mice and humans. *Nature* **371**:802-806.
23. Halbert, C. L., M. L. Aitken, and A. D. Miller. 1996. Retroviral vectors efficiently transduce basal and secretory airway epithelial cells *in vitro* resulting in persistent gene expression in organotypic culture. *Hum. Gene Ther.* **7**:1871-1881.
24. Halbert, C., G. W. Demers, and D. A. Galloway. 1991. The E7 gene of human papillomavirus type 16 is sufficient for immortalization of human epithelial cells. *J. Virol.* **65**:473-478.
25. Hecht, S. J., J. O. Carlson, and J. C. DeMartini. 1994. Analysis of a type D retroviral capsid gene expressed in ovine pulmonary carcinoma and present in both affected and unaffected sheep genomes. *Virology* **202**:480-484.
26. Hecht, S. J., J. M. Sharp, and J. C. DeMartini. 1996. Retroviral aetiopathogenesis of ovine pulmonary carcinoma: a critical appraisal. *Br. Vet. J.* **152**:395-409.
27. Hecht, S. J., K. Stedman, J. Carlson, and J. C. DeMartini. 1996. Distribution of endogenous type B and D retroviral sequences in ungulates and other mammals. *Proc. Natl. Acad. Sci. USA* **93**:3297-3302.
28. Heiber, M., J. M. Docherty, G. Shah, T. Nguyen, R. Cheng, H. H. Q. Heng, A. Marchese, L.-C. Tsui, X. Shi, S. R. George, and B. F. O'Dowd. 1995. Isolation of three novel human genes encoding G protein-coupled receptors. *DNA Cell Biol.* **14**:25-35.
29. Heiber, M., A. Marchese, T. Nguyen, H. H. Q. Heng, S. R. George, and B. F. O'Dowd. 1996. A novel human gene encoding a G-protein-coupled receptor (GPR15) is located on chromosome 3. *Genomics* **32**:462-465.
30. Holland, M. J., M. Palmarini, M. Garcia-Goti, L. Gonzalez, I. McKendrick, M. de las Heras, and J. M. Sharp. 1999. Jaagsiekte retrovirus is widely distributed in both T and B lymphocytes and in mononuclear phagocytes of the sheep with naturally and experimentally acquired pulmonary adenomatosis. *J. Virol.* **73**:4004-4008.
31. Hyde, S., D. Gill, C. Higgins, A. Trezise, L. Macvinish, A. Cuthbert, R. Ratcliff, M. Evans, and W. Colledge. 1993. Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy. *Nature* **362**:250-255.
32. Ives, J. C., P. A. Buffler, and S. D. Greenberg. 1983. Environmental associations and histopathological patterns of carcinoma of the lung: the challenge and dilemma in epidemiological studies. *Am. Rev. Respir. Dis.* **128**:195-209.
33. Kaebbling, M., R. Eddy, T. B. Shows, N. G. Copeland, D. J. Gilbert, N. A. Jenkins, H. P. Klinger, and B. O'Hara. 1991. Localization of the human gene allowing infection by gibbon ape leukemia virus to human chromosome region 2q11-q14 and to the homologous region on mouse chromosome 2. *J. Virol.* **65**:1743-1747.
34. Loetscher, M., A. Amara, E. Oberlin, N. Brass, D. F. Legler, P. Loetscher, M. D'Apuzzo, E. Meese, D. Rousset, J.-L. Virelizier, M. Baggiolini, F. Arenzana-Seisdedos, and B. Moser. 1997. TYMSTR, a putative chemokine receptor selectively expressed in activated T cells, exhibits HIV-1 coreceptor function. *Cell Biol.* **7**:652-660.
35. Maho, A., A. Bensimon, G. Vassart, and M. Parmentier. 1999. Mapping of the CCXCR1, CX3CR1, CCBP2 and CCR9 genes to the CCR cluster within the 3p21.3 region of human genome. *Cytogenet. Cell Genet.* **87**:265-268.
36. Marchese, A., J. M. Docherty, T. Nguyen, M. Heiber, R. Cheng, H. H. Q. Heng, L.-C. Tsui, X. Shi, S. R. George, and B. F. O'Dowd. 1994. Cloning of human genes encoding novel G protein-coupled receptors. *Genomics* **23**:609-618.
37. Martin, W. B., F. M. Scott, J. M. Sharp, K. W. Angus, and M. Norval. 1976. Experimental production of sheep pulmonary adenomatosis (jaagsiekte). *Nature* **264**:183-185.
38. Miller, A. D., and F. Chen. 1996. Retrovirus packaging cells based on 10A1 murine leukemia virus for production of vectors that use multiple receptors for entry. *J. Virol.* **70**:5564-5571.
39. Miller, A. D., J. V. Garcia, N. von Suhr, C. M. Lynch, C. Wilson, and M. V. Eiden. 1991. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. *J. Virol.* **65**:2220-2224.
40. Miller, A. D., and G. J. Rosman. 1989. Improved retroviral vectors for gene transfer and expression. *Biotechniques* **7**:980-990.
41. Miller, A. D., and G. Wolgamot. 1997. Murine retroviruses use at least six different receptors for entry into *Mus dunni* cells. *J. Virol.* **71**:4531-4535.
42. Miller, D. G., R. H. Edwards, and A. D. Miller. 1994. Cloning of the cellular receptor for amphotropic murine retroviruses reveals homology to that for gibbon ape leukemia virus. *Proc. Natl. Acad. Sci. USA* **91**:78-82.
43. O'Dowd, B. F., M. Heiber, A. Chan, H. H. Q. Heng, L.-C. Tsui, J. L. Kennedy, X. Shi, A. Petronis, S. R. George, and T. Nguyen. 1993. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* **136**:355-360.
44. Palmarini, M., C. Cousens, R. G. Dalziel, J. Bai, K. Stedman, J. C. DeMartini, and J. M. Sharp. 1996. The exogenous form of jaagsiekte retrovirus is specifically associated with a contagious lung cancer of sheep. *J. Virol.* **70**:1618-1623.
45. Palmarini, M., P. Dewar, M. de las Heras, N. F. Inglis, R. G. Dalziel, and J. M. Sharp. 1995. Epithelial tumor cells in the lungs of sheep with pulmonary adenomatosis are major sites of replication for jaagsiekte retrovirus. *J. Gen. Virol.* **76**:2731-2737.
46. Palmarini, M., H. Fan, and J. M. Sharp. 1997. Sheep pulmonary adenomatosis: a unique model of retrovirus-associated lung cancer. *Trends Microbiol.* **5**:478-483.

47. **Palmarini, M., M. J. Holland, C. Cousens, R. G. Dalziel, and J. M. Sharp.** 1996. Jaagsiekte retrovirus establishes a disseminated infection of the lymphoid tissues of sheep affected by pulmonary adenomatosis. *J. Gen. Virol.* **77**:2991–2998.
48. **Palmarini, M., J. M. Sharp, M. de las Heras, and H. Fan.** 1999. Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *J. Virol.* **73**:6964–6972.
49. **Perk, K., and I. Hod.** 1982. Sheep lung carcinoma: an endemic analogue of a sporadic human neoplasm. *JNCI* **69**:747–749.
50. **Pickles, R. J., D. McCarty, H. Matsui, P. J. Hart, S. H. Randell, and R. C. Boucher.** 1998. Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer. *J. Virol.* **72**:6014–6023.
51. **Quade, K.** 1979. Transformation of mammalian cells by avian myelocytomatosis virus and avian erythroblastosis virus. *Virology* **98**:461–465.
52. **Rasko, J. E. J., J.-L. Battini, R. J. Gottschalk, I. Mazo, and A. D. Miller.** 1999. The RD114/simian type D retrovirus receptor is a neutral amino acid transporter. *Proc. Natl. Acad. Sci. USA* **96**:2129–2134.
53. **Rosenfeld, M. A., K. Yoshimura, B. C. Trapnell, K. Yoneyama, E. R. Rosenthal, W. Dalemans, M. Fukayama, J. Bargon, L. E. Stier, S. Stratford-Perricaudet, M. Perricaudet, M. W. B. Guggino, A. Pavirani, J. Lecocq, and R. G. Crystal.** 1992. *In vivo* transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell* **68**:143–155.
54. **Samson, M., A. L. Edinger, P. Stordeur, J. Rucker, V. Verhasselt, M. Sharon, C. Govaerts, C. Mollereau, G. Vassart, R. W. Doms, and M. Parmentier.** 1998. ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* **28**:1689–1700.
55. **Samson, M., P. Soularue, G. Vassart, and M. Parmentier.** 1996. The genes encoding the human CC-chemokine receptors CC-CCR1 to CC-CCR5 (CMKBR1-CMKBR5) are clustered in the p21.3-p24 region of chromosome 3. *Genomics* **36**:522–526.
56. **Samson, M., P. Stordeur, O. Labbe, P. Soularue, G. Vassart, and M. Parmentier.** 1996. Molecular cloning and chromosomal mapping of a novel human gene, ChemR1, expressed in T lymphocytes and polymorphonuclear cells and encoding a putative chemokine receptor. *Eur. J. Immunol.* **26**:3021–3028.
57. **Stewart, E. A., K. B. McKusick, A. Aggarwal, E. Bajorek, S. Brady, A. Chu, N. Fang, D. Hadley, M. Harris, S. Hussain, R. Lee, A. Maratukulam, K. O'Connor, S. Perkins, M. Piercy, F. Qin, T. Reif, C. Sanders, X. She, W.-L. Sun, P. Tabar, S. Voyticky, S. Cowles, J.-B. Fan, C. Mader, J. Quackenbush, R. M. Myers, and D. R. Cox.** 1997. An STS-based radiation hybrid map of the human genome. *Genome Res.* **7**:422–433.
58. **Tajima, Y., K. Tashiro, and D. Camerini.** 1997. Assignment of the possible HTLV receptor gene to chromosome 17q21-q23. *Somat. Cell Mol. Genet.* **23**:225–227.
59. **Verwoerd, D. W., A. L. Williamson, and E. M. De Villiers.** 1980. Aetiology of jaagsiekte: transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. *Onderstepoort J. Vet. Res.* **47**:275–280.
60. **Wei, C.-M., M. Gibson, P. G. Spear, and E. M. Scolnick.** 1981. Construction and isolation of a transmissible retrovirus containing the *src* gene of Harvey murine sarcoma virus and the thymidine kinase gene of herpes simplex virus type 1. *J. Virol.* **39**:935–944.
61. **York, D. F., R. Vigne, D. W. Verwoerd, and G. Querat.** 1992. Nucleotide sequence of the jaagsiekte retrovirus, an exogenous and endogenous type D and B retrovirus of sheep and goats. *J. Virol.* **66**:4930–4939.
62. **Zeitlin, P. L., L. Lu, J. Rhim, G. Cutting, G. Stetten, K. A. Kieffer, R. Craig, and W. B. Guggino.** 1991. A cystic fibrosis bronchial epithelial cell line: immortalization by Adeno-12-SV40 infection. *Am. J. Respir. Cell Mol. Biol.* **4**:313–319.
63. **Zsengeller, Z. K., C. Halbert, A. D. Miller, S. E. Wert, J. A. Whitsett, and C. J. Bachurski.** 1999. Keratinocyte growth factor stimulates transduction of the respiratory epithelium by retroviral vectors. *Hum. Gene Ther.* **10**:341–353.