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Jaagsiekte Sheep Retrovirus Envelope Efficiently Pseudotypes Human Immunodeficiency Virus Type 1-Based Lentiviral Vectors

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Jaagsiekte sheep retrovirus (JSRV) infects lung epithelial cells in sheep, and oncoretroviral vectors bearing JSRV Env can mediate transduction of human cells, suggesting that such vectors might be useful for lung-directed gene therapy. Here we show that JSRV Env can also efficiently pseudotype a human immunodeficiency virus type 1-based lentiviral vector, a more suitable vector for transduction of slowly dividing lung epithelial cells. We created several chimeric Env proteins that, unlike the parental Env, do not transform rodent fibroblasts but are still capable of pseudotyping lentiviral and oncoretroviral vectors.

The ability of lentiviral vectors to transduce nondividing cells and maintain long-term expression of transgenes (36, 37) has made them attractive tools for treatment of a variety of genetic diseases, including cystic fibrosis (CF). CF occurs in 1 in 3,200 Caucasian births and is caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein (45). Strategies to treat CF have employed vectors derived from adenovirus (18, 21), adeno-associated virus (1, 3, 20), and oncoretroviruses (15, 51), but the utility of these vectors is limited by either host immune response, restricted packaging capacity, or low transduction efficiency, respectively.

The human immunodeficiency virus type 1 (HIV-1) core can be pseudotyped by a variety of retroviral envelope proteins, such as those of murine leukemia viruses (MLVs) (25, 29), human T-cell leukemia virus type 1 (24), and avian leukosis-sarcoma virus subgroup A (25), as well as by some nonretroviral envelopes, including vesicular stomatitis virus G protein (VSV-G) (35, 37), lymphocytic choriomeningitis virus glycoprotein (4), Mokola virus and rabies virus G proteins (35), and Ebola virus (Zaire) glycoprotein (23). HIV-1 lentiviral vectors, most pseudotyped with VSV-G, have been successfully used for transduction of a variety of cells, including neurons (5, 37), retinal cells (34, 47), and $CD34^+$ hematopoietic stem cells (6, 14, 33, 46). Interestingly, the VSV-G-pseudotyped HIV-1 vectors have been shown to inefficiently transduce differentiated airway epithelial cells in vivo (17). Other envelope proteins, in particular those from viruses that predominantly infect the respiratory system, such as influenza virus and respiratory syncytial virus, have also been used to pseudotype HIV-1 vectors but failed to promote airway gene transfer (23). Given that glycosylphosphatidylinositol (GPI)-anchored proteins are enriched on the apical surfaces of polarized epithelial cells (26), it seems reasonable to hypothesize that a vector utilizing a GPI-anchored protein as its cellular receptor should yield higher transduction efficiency and would be ideal for lung airway gene transfer. This notion is supported by a recent report that replacement of the transmembrane domain and cytoplasmic tail of adenovirus receptor CAR with a GPI anchor facilitates adenovirus-mediated gene transfer to lung airway epithelia (49). Recently, Kobinger et al. (23) also demonstrated that HIV-1 lentiviral vectors bearing the Env protein of Ebola virus Zaire strain, which uses the GPI-anchored folate receptor-α as an entry factor, efficiently transduces airway epithelial cells in vitro and in human bronchial xenografts.

JSRV is a simple betaretrovirus that infects lower airway epithelial cells, predominantly type II pneumocytes and Clara cells (16), leading to the development of a contagious lung cancer in sheep known as ovine pulmonary adenocarcinoma (11, 39). Given lung-specific tropism of JSRV and its ability to replicate efficiently in the presence of lung fluid containing surfactants and proteases (12, 31, 48), it was previously hypothesized that JSRV might provide an effective tool for lung airway gene therapy (41). The identification of hyaluronidase 2 (Hyal2) as a GPI-anchored cell surface protein that serves as the receptor for jaagsiekte sheep retrovirus (JSRV) (42) renders this hypothesis even more attractive. Indeed, it has been demonstrated that JSRV Env protein efficiently pseudotypes the Moloney MLV (MoMLV) oncoretroviral vectors, and the JSRV pseudotypes efficiently transduce most human cell lines examined, including human epithelial cells (41, 42). We further showed that MoMLV oncoretrovirus vectors bearing the JSRV envelope protein are stable during treatment with lung surfactant, centrifugation, and freeze-thaw cycling, in contrast to an identical vector bearing an amphotropic MLV Env that was inactivated by these treatments (9).

Studies from our lab and others showed that JSRV Env protein can transform fibroblast cell lines derived from mice, rats, and chickens (2, 30, 42), as well as an immortalized human bronchioalveolar epithelial cell line (BEAS-2B) (10), indicating that JSRV Env protein plays a key role in ovine tumorigenesis. In fibroblasts the transforming activity was mediated by the cytoplasmic tail of Env, while in BEAS-2B cells interaction of Env with Hyal2 played a key role in transformation. The oncogenic properties of JSRV Env pose a significant challenge for use of JSRV vectors in human clinical trials, and development of JSRV vectors devoid of oncogenic activity while still capable of transducing human lung epithelial cells is

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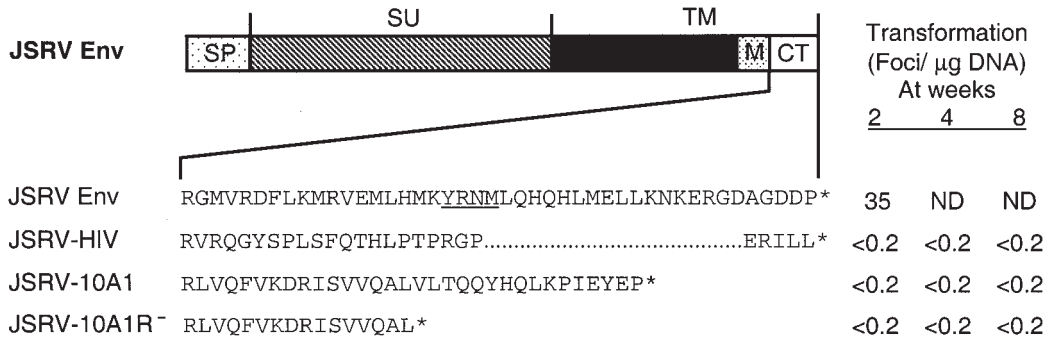

FIG. 1. JSRV Env constructs. The full-length JSRV Env structure (GenBank accession no. AF357971) is schematically presented, with the cytoplasmic tail (CT) sequence shown under the structure. The YXXM motif is underlined. For chimeras, the cytoplasmic tail of JSRV Env was replaced by that of HIV-1 (JSRV-HIV; dots represent the HIV-1 sequence omitted), 10A1 MLV (JSRV-10A1), or 10A1 MLV lacking the R peptide (JSRV-10A1R$^-$), respectively. Shown at right are the transforming activities of corresponding JSRV Env constructs examined at the indicated weeks following DNA transfection. SU, surface subunit; TM, transmembrane subunit; SP, signal peptide; M, membrane-spanning domain; ND, not done. *, stop codon.

highly desirable. We have focused on alteration of the Env cytoplasmic tail to reduce its transforming activity, since it is likely that transforming activity associated with Env binding to Hyal2 cannot be reduced without compromising Env-mediated cell entry.

Here we demonstrate that the JSRV Env and a chimeric JSRV-HIV Env with the cytoplasmic domain of JSRV replaced with that of HIV-1 can efficiently pseudotype HIV-1 lentiviral vectors. Vectors bearing the chimeric Env retain the ability to transduce BEAS-2B immortalized human bronchial epithelial cells (43) and immortalized epithelial cells from a CFTR-deficient patient (CF16) (19). Moreover, these vectors are resistant to treatment with repeated freeze-thaw cycling and ultracentrifugation. These results suggest that lentiviral vectors bearing JSRV Env may be useful for airway gene transfer for treatment of human lung disorders such as CF.

Construction of JSRV Env expression plasmids. The full-length JSRV Env used in this study (GenBank accession no. AF357971) (11) encodes a 615-amino-acid protein with predicated molecular masses of ~50 and ~35 kDa for the surface and transmembrane subunits, respectively (Fig. 1). To generate JSRV Env expression constructs, we isolated the full-length JSRV Env sequence by PCR as previously described (28). Three JSRV Env chimeras, in which the cytoplasmic tail of JSRV Env was replaced by that of HIV-1 (LAI strain, GenBank accession no. K02013), 10A1 amphotropic MLV (GenBank accession no. M33470), or 10A1 lacking the R peptide, referred to as JSRV-HIV, JSRV-10A1, and JSRV-10A1R$^-$, respectively (Fig. 1), were generated by overlapping PCR as described (28). PCR products were cloned into pCI-neo (Promega Corporation, Madison, Wis.), an expression vector with a human cytomegalovirus (CMV) immediate early promoter. For each construct, at least two independent clones with confirmed sequences were examined for transforming activity and pseudotyping of lentiviral or oncoretroviral vectors.

JSRV Env can pseudotype an HIV-1 vector. To investigate whether JSRV Env could pseudotype an HIV-1 lentiviral vector, we cotransfected 293T cells with plasmids encoding the JSRV Env protein (pCI-Jenv) and HIV-1 Gag-Pol proteins (pCMVΔR8.2) (37) and a plasmid containing an HIV-1-based vector encoding human placental alkaline phosphatase (AP) (AP was inserted in place of LacZ in the pHr′CMVLacZ vector (37) (to make pHr′CMVAP). Two to 3 days after DNA transfection, medium from the transfected cells was harvested and filtered through 0.45-μM-pore-size filters and was used for infection of cells. As a positive control, we made a VSV-G-pseudotype lentiviral vector by using the same procedure except that the JSRV Env plasmid was replaced with a VSV-G expression plasmid (pMD.G) (37). JSRV Env efficiently pseudotyped the HIV-1-based lentiviral vector, giving a titer on HTX cells (a diploid subclone of HT-1080 human fibrosarcoma cells) of 10^4 AP$^+$ focus-forming units (FFU)/ml, only threefold lower than that of the VSV-G-pseudotype lentiviral vector (Table 1). The titer of the JSRV-pseudotype lentiviral vector on various human cell lines ranged from 1×10^4 to 5×10^4 AP$^+$ FFU/ml.

For comparison we also made JSRV-Env- and VSV-G-pseudotype oncoretroviral vectors by transfection of 293T cells that make MoMLV Gag-Pol proteins and that contain an integrated MoMLV vector encoding AP (LAPSN) (32) with plasmids encoding JSRV Env or VSV-G. Virus was prepared as described above for the lentiviral vectors. Lentiviral and oncoretroviral vectors bearing JSRV Env exhibited similar titers for the human cell lines tested, while the titer for the oncoret-

TABLE 1. Host ranges and titers of HIV-1 lentiviral and MoMLV oncoretroviral vectors bearing the JSRV Env protein[a]

Env source	Target cells	Species	Titer (AP$^+$ FFU/ml) for indicated vector:	
			Lentiviral	Oncoretroviral
JSRV	HTX	Human	1.1×10^4	4.2×10^4
	293	Human	5.0×10^4	3.8×10^4
	BEAS-2B	Human	2.9×10^4	2.5×10^4
	SSF	Sheep	7.5×10^4	1.0×10^5
	NIH 3T3	Mouse	<5	<5
	208F	Rat	<5	<5
VSV-G	HTX	Human	3.4×10^4	1.5×10^6

[a] Titers of viral stocks were determined by infection of cells seeded the day before at 10^5 cells per well of 6-well plates with serially diluted virus, and AP$^+$ foci were counted 3 days after infection. The values shown are the means of the results of at least two experiments, and the variation in the results of the experiments was no more than 10%.

roviral vector bearing VSV-G was 40-fold higher than that of the lentiviral vector bearing VSV-G (Table 1). Both lentiviral and oncoretroviral vectors bearing JSRV Env exhibited higher titers for the sheep skin fibroblasts (SSFs) than for human cells and show undetectable titers for mouse and rat cells (Table 1), which is consistent with previous results (13, 27, 41).

JSRV Env mutants that do not transform rodent fibroblasts can pseudotype both lentiviral and oncoretroviral vectors. We created three JSRV Env chimeras in which the cytoplasmic tail of JSRV Env was replaced by that of either HIV-1, 10A1 amphotropic MLV, or 10A1 lacking the R peptide (Fig. 1). The R peptide is a 16-amino-acid sequence present at the carboxy termini of most gammaretroviral (e.g., MLVs, gibbon ape leukemia virus, spleen necrosis virus, and pig endogenous viruses) and some betaretroviral (Mason-Pfizer monkey virus) and lentiviral (equine anemia virus) envelope proteins. The R peptide has been shown to play an inhibitory role in cell-cell fusion and infectivity (22, 40, 44, 50). In this study, we wished to determine if deletion of the R peptide of MLV Env would have any effect on the ability of chimeric JSRV Env proteins to pseudotype HIV-1 and/or MoMLV vectors.

We first examined the transforming activities of these JSRV Env chimeras in 208F cells using a previously described method (28). Briefly, 10-μg plasmid DNA encoding an individual Env protein was transfected into 5×10^5 208F cells by using a modified calcium phosphate coprecipitation method (7). Cells were trypsinized and split at a ratio of 1:3 the next day. Cells were fed every 3 to 4 days, and transforming activities were examined. None of the JSRV Env chimeras induced focus formation at 2, 4, or 8 weeks posttransfection, in sharp contrast to the wild type (JSRV Env), which showed robust transforming activity at 2 weeks after transfection (Fig. 1). These results are consistent with previous data that chimeric JSRV-HIV and JSRV-10A1 Env proteins expressed in the backbone of pSX2neo, an MoMLV long terminal repeat promoter-driven mammalian expression vector (32), were unable to induce cell transformation in 208F cells in a prolonged transformation assay (28). The relatively low transforming activity of wild-type JSRV Env in this study (~35 FFU of DNA/μg) (Fig. 1) compared to that previously reported (~120 FFU of DNA/μg) (28) is likely due to the relatively weak activity of the CMV promoter in rodent cells compared to that of the retroviral long terminal repeat used in the previous study.

We next tested the ability of these nontransforming JSRV Env constructs to pseudotype the HIV-1 lentiviral and MoMLV oncoretroviral vectors. The JSRV lentiviral pseudotypes bearing each Env chimera were generated by using the same cotransfection method as described above for the wild-type JSRV Env. For these comparisons, production of lentiviral and oncoretroviral vectors was performed in parallel for each independent experiment, and titers of both vectors were determined on HTX cells at the same time. As shown in Table 2, all nontransforming JSRV Env chimeras were able to pseudotype the HIV-1 lentiviral and MoMLV oncoretroviral vectors, albeit with varied titers. Among them, the chimeric JSRV-HIV-pseudotyped lentiviral vectors reproducibly exhibited the highest titers. These titers were only slightly lower than those of identical vectors pseudotyped by the wild-type JSRV Env.

Interestingly, we found that the MoMLV oncoretroviral vector bearing the same JSRV-HIV chimera had a titer ~10- to 100-fold lower than that of an identical oncoretroviral vector pseudotyped with the wild-type JSRV Env. Given comparable transfection efficiency between JSRV-HIV chimera and JSRV Env wild type on 293 cells (as determined by the numbers of G418-positive colonies; data not shown) and equivalent titers obtained for lentiviral and oncoretroviral vectors bearing the same wild-type JSRV Env (Table 2), it appears that the cytoplasmic tail of HIV-1 Env fused at the carboxy terminus of the extracellular domain of JSRV Env protein is more suitable for the production of the HIV-1 lentiviral vectors than for MoMLV vectors.

TABLE 2. Titers of HIV-1 lentiviral and MoMLV oncoretroviral vectors pseudotyped with chimeric JSRV envelopes[a]

Env construct[b]	Lentiviral vector titer (AP^+ FFU/ml) on indicated cell type:		Oncoretroviral vector titer (AP^+ FFU/ml) on indicated cell type:	
	HTX	SSF	HTX	SSF
JSRV Env	1.3×10^4	7.8×10^4	2.9×10^4	1.1×10^5
JSRV-HIV	7.7×10^3	4.7×10^4	1.7×10^2	1.3×10^4
JSRV-10A1	8.6×10^2	8.0×10^3	1.1×10^2	3.7×10^2
JSRV-10A1R$^-$	1.3×10^3	7.0×10^3	2.4×10^3	9.2×10^3

[a] The experimental approaches used were the same as those described in Table 1 except that wild-type or nontransforming JSRV Env constructs were used for pseudotyping. The values shown are the means of the results of at least two experiments, and the variation in the results of the experiments was no more than 10%.

[b] See the legend to Fig. 1 for construct nomenclature.

Effect of R peptide on pseudotyping. The effect of the R peptide on Env incorporation onto the oncoretroviral and lentiviral vectors remains elusive (8). In this study, we investigated this issue by creating two chimeras between JSRV and MLV Env proteins, one with R peptide intact (JSRV-10A1), and another with R peptide removed by mutagenesis (JSRV-10A1R$^-$) (Fig. 1). While the titers of lentiviral vectors bearing JSRV-10A1 or JSRV-10A1R$^-$ were similar, the MoMLV oncoretroviral vector bearing JSRV-10A1R$^-$ showed a >10-fold-higher titer than an otherwise identical vector bearing JSRV-10A1 (Table 2). These results suggest that the R peptide of MLV Env may inhibit the efficiency of chimeric JSRV-10A1 Env protein incorporation into the MoMLV oncoretroviral vector, but it plays a less important role in pseudotyping of the lentiviral vector. Further clarification of this issue requires detailed examination of Env protein expression as well as the interactions of these proteins with HIV-1 or MoMLV cores in the virus-producing cells. It should be added, however, that we did not observe any syncytia formation for any of the JSRV Env constructs, including the R-peptide-deficient JSRV-10A1R$^-$.

Transduction of JSRV vectors is restricted to cells expressing Hyal2, the JSRV receptor. The lack of specific antibody against JSRV Env protein (in particular the extracellular domain common to all constructs) made it impossible to directly examine the incorporation of JSRV Env protein into lentiviral or oncoretroviral vectors. We therefore addressed this issue indirectly by measuring transduction by the JSRV vectors in cells expressing a functional JSRV receptor Hyal2 and in cells that do not. As shown in Table 3, all JSRV pseudotypes, including those bearing the nontransforming chimeras, transduced only NIH 3T3 or 208F cells expressing a functional

TABLE 3. Transduction by HIV-1 lentiviral and MoMLV oncoretroviral vectors pseudotyped with JSRV Env is restricted to cells expressing the JSRV receptor Hyal2[a]

Vector type	Env construct	Vector titer (AP^+ FFU/ml) for indicated target cells:			
		NIH 3T3	NIH 3T3/LL2SN	208F	208F/LL2SN
Lentiviral	JSRV	<5	2.8×10^3	<5	1.5×10^2
	JSRV-HIV	<5	3.7×10^3	<5	9.0×10^2
	JSRV-10A1	<5	2.5×10^2	<5	2.5×10^2
	JSRV-10A1R$^-$	<5	3.0×10^2	<5	3.5×10^2
	VSV-G	5.8×10^4	6.4×10^4	9.0×10^3	1.3×10^4
Oncoretroviral	JSRV	<5	2.8×10^5	<5	1.5×10^5
	JSRV-HIV	<5	2.8×10^4	<5	9.0×10^2
	JSRV-10A1	<5	8.0×10^2	<5	2.5×10^2
	JSRV-10A1R$^-$	<5	1.8×10^4	<5	8.5×10^3
	VSV-G	1.4×10^6	1.6×10^6	8.6×10^5	8.0×10^5

[a] The methods used were the same as those described in Table 1. The titers shown are the means of the results of two experiments and varied by <10%. NIH 3T3/LL2SN and 208F/LL2SN cells are NIH 3T3 and 208F cells that were transduced with a retroviral vector that expresses human Hyal2.

receptor, human Hyal2, but did not transduce the parental NIH 3T3 or 208F cells, whose endogenous rodent Hyal2 protein does not function as a JSRV receptor (27, 42). In contrast and as expected, the VSV-G-pseudotyped HIV-1 or MoMLV vector transduced NIH 3T3 and 208F cells and their derivatives expressing human Hyal2 (Table 3) at about the same rate, suggesting that the Hyal2 restrictions of NIH 3T3 and 208F cells are indeed specific for JSRV Env. Taken together, these data indicate that JSRV Env protein and its chimeras are present on the HIV-1 lentiviral or MoMLV oncoretroviral particles and mediate vector entry into the target cells.

Transduction of primary and cultured human lung epithelial cells by JSRV lentiviral vectors. As the first step in testing whether the JSRV pseudotypes, in particular the lentiviral vectors bearing the nontransforming JSRV Env chimeras, would be useful for human airway gene transfer, we examined their abilities to transduce BEAS-2B and CF16 lung epithelial cells. BEAS-2B cells are human bronchial epithelial cells immortalized by an adenovirus-12/SV40 hybrid virus (43), and CF16 cells are nasal epithelial cells from a CFTR-deficient patient immortalized by expression of human papillomavirus 16 E6 and E7 genes (19). As shown in Table 4, all lentiviral and oncoretroviral vectors bearing the nontransforming JSRV Env chimeras were capable of transducing both BEAS-2B and CF16 cells. The lentiviral vector bearing the JSRV-HIV Env exhibited titers almost as high as those bearing the wild-type JSRV Env on both cell types (Table 4). Consistent with results shown above, the MoMLV oncoretroviral vectors (but not the HIV-1 lentiviral vector) bearing JSRV-10A1R$^-$ showed titers of approximately 1 log greater on BEAS-2B and CF16 cells than those of an identical MoMLV vector bearing JSRV-10A1 Env (Table 4).

We next examined JSRV lentiviral and JSRV oncoretroviral vector transduction of primary human tracheal epithelial cells. While human tracheal epithelial cells were resistant to transduction by all JSRV MoMLV pseudotypes (wild type and chimeras), they were transduced by the corresponding JSRV lentiviral pseudotypes, albeit with low efficiencies ($\sim 10^2$ FFU/ml for JSRV-HIV Env without concentration).

Stability of lentiviral vectors. Previously, we showed that an MoMLV vector bearing the JSRV Env is stable during treatment with surfactant, freeze-thaw cycling, and ultracentrifugation (9). Here we tested the stability of JSRV HIV-1 lentiviral vectors by subjecting them to repeated cycles of freezing to −80°C and thawing to 37°C. After five rounds of freeze-thaw cycles, the titers of these JSRV pseudotypes for HTX cells were essentially unchanged (data not shown). The JSRV lentiviral pseudotypes can also be concentrated to a higher titer by ultracentrifugation without significant loss of activity (data not shown).

Discussion. Despite much effort and the progress that has been made in the past decade, lung airway gene therapy for treatment of CF is still not available. This study was designed to explore if JSRV can provide an alternative approach for airway gene therapy by using HIV-1 lentiviral vectors. We demonstrate that not only the wild-type JSRV Env but also several Env chimeras that do not transform rodent fibroblasts can pseudotype an HIV-1 lentiviral vector. Among the nontransforming chimeras, JSRV-HIV, in which the cytoplasmic tail of JSRV Env is replaced with that of HIV-1, exhibits the highest ability to pseudotype the HIV-1 vector, and the resulting JSRV lentiviral vectors can transduce human lung epithelial cells. Therefore, the JSRV-HIV chimeric Env appears to be the best candidate for lung airway gene transfer in vivo.

TABLE 4. Transduction of human lung airway epithelial cells by HIV-1 lentiviral and MoMLV oncoretroviral vectors bearing JSRV Env proteins[a]

Env construct	Titer (AP^+ FFU/ml) for indicated target cells:			
	BEAS-2B		CF16	
	Lentiviral vector	Oncoretroviral vector	Lentiviral vector	Oncoretroviral vector
JSRV Env	3.3×10^4	2.8×10^4	9.0×10^3	1.4×10^4
JSRV-HIV	1.8×10^4	4.0×10^2	7.2×10^3	2.9×10^2
JSRV-10A1	1.8×10^3	3.0×10^2	6.2×10^2	51
JSRV-10A1R$^-$	3.8×10^3	2.9×10^3	6.1×10^3	4.5×10^2
VSV-G	5.9×10^4	1.5×10^6	8.8×10^3	1.2×10^5

[a] Experimental approaches were the same as those described in Table 1. The titers on BEAS-2B human lung epithelial cells (43) and CF16 cells established from a CF patient (18) were determined and shown. The values shown are the means of the results of two independent experiments, with variations of no more than 10%.

While JSRV Env wild type and its nontransforming chimeras can pseudotype HIV-1 lentiviral vectors, the titers of these vectors ($\sim 10^4$ FFU/ml without concentration) are relatively low. However, an identical HIV-1 lentiviral vector bearing VSV-G also shows low titers on all cell types examined (Tables 1, 3, and 4), suggesting that the relatively low titers of JSRV lentiviral pseudotypes are typical of our production system and/or assay conditions. In these studies we used an early HIV vector design (36) that exhibits relatively low titer in comparison to more recent designs. Currently, we are working on conditions to improve the titers of these JSRV lentiviral pseudotypes. Since expression of the wild-type and chimeric JSRV Env proteins is not toxic to cells, we should be able to make stable packaging cell lines for production of these vectors and in this way increase the vector titer.

We initially expected that some JSRV Env point mutants, in particular those involving the cytoplasmic YXXM motif (Fig. 1) that has been shown to be critical for rodent cell transformation (38), would be the best candidates for JSRV vector development, given that their sequences have the closest similarities to those of the wild type. Indeed, we have created a series of such mutants and tested their abilities to pseudotype HIV-1 lentiviral and MoMLV oncoretroviral vectors. While all these mutants were able to pseudotype the HIV-1 lentiviral vector, their titers were generally low (data not shown). Furthermore, all these JSRV Env mutants were still able to induce transformed foci in a prolonged cell transformation assay (27; data not shown). Therefore, we have excluded these mutants from this study since they would be less appropriate for gene therapy applications.

While the extracellular domain of the JSRV Env protein does not induce cell transformation in rodent and chicken fibroblasts (2, 28, 38), it remains to be determined if this region is competent in eliciting oncogenic transformation of human epithelial cells. This concern is highlighted by the recent demonstration that JSRV Env protein can transform BEAS-2B human lung epithelial cells by interaction with Hyal2 and inhibition of Hyal2 tumor suppressor activity (10). If the extracellular domain alone can transform epithelial cells, it may be difficult to dissociate this transforming activity of Env from its ability to mediate cell entry by using the Hyal2 receptor.

Since murine cells are not transduced by JSRV vectors, due to the lack of a functional receptor, we are currently unable to examine the transduction efficiency of these JSRV lentiviral pseudotypes in mice. Ovine cells are highly transducible by JSRV vectors, but sheep are expensive and difficult to handle experimentally. One feasible approach is to generate transgenic mice expressing human Hyal2 and to evaluate the JSRV lentiviral pseudotypes on them. Alternatively, we will test these JSRV vectors in human bronchial xenografts. Results from these studies may provide important information for us to use in further evaluation the JSRV lentiviral vectors in human airway gene transfer.

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