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Differential sensitivity of porcine endogenous retrovirus to APOBEC3-mediated inhibition

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Abstract Pigs are considered to be suitable xenotransplantation organ donors. However, the risk of pathogen transmission from pigs to humans is a major concern in the transplantation of porcine tissues. The porcine endogenous retroviruses (PERVs) PERV-A, PERV-A/C, and PERV-B can infect human cells, but PERV-C is an ecotropic virus infecting only pig cells. Thus, several strategies have been proposed to reduce PERV transmission in xenograft recipients. Human APOBEC3G (huA3G) is a single-strand DNA cytosine deaminase, which inactivates the coding capacity of the virus by deamination of cDNA cytosines to uracils. This reaction occurs within the (−) DNA strand during reverse transcription, resulting in a G-to-A mutation in the (+) strand. While recent data have shown that PERV-B is severely inhibited by huA3G and porcine A3Z2-Z3 (poA3F) in a pseudotype assay, little is known about PERV-C. Here, we compare the antiretroviral activities of huA3G, huA3F and poA3Z2-Z3 against PERV-C. Our data show that APOBEC3 was packaged into PERV-C particles and inhibited PERV-C replication in a dose-dependent manner. PERV-C infectivity was strongly inhibited by poA3Z2-Z3, but it did not markedly reduce PERV-B infectivity. This suggests that PERV-C Gag interacts efficiently with poA3Z2-Z3. In addition, we constructed stably huA3G- and poA3Z2-Z3-expressing 293-PERV-PK-CIRCE cells (human 293 cells infected with PK15-derived PERVs) to examine whether PERV is resistant to poA3Z2-Z3 in a virus-spreading assay. The stably expressed huA3G and poA3Z2-Z3 were more packaging-competent than transiently expressed APOBEC3 proteins. These results suggest that poA3Z2-Z3 can inhibit PERV replication in a pseudotype assay as well as in a virus-spreading assay.

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Introduction

Porcine endogenous retroviruses (PERVs) are members of the family *Retroviridae*, genus *Gammaretrovirus*. They are closely related to mammalian type-C retroviruses, such as gibbon ape leukemia virus and murine leukemia virus. Two classes of infectious human-tropic replication-competent PERVs (polytropic PERV-A and PERV-B) and one class of ecotropic PERV (PERV-C) are known [1–4]. PERV-C is capable of infecting porcine cells only. In addition to these three classes of PERVs, recombinants between ecotropic PERV-C and human-tropic PERV-A have been detected in porcine cells [5]. One of the recombinant PERV-A/C viruses was found to be 500-fold more infectious than the prototype PERV-A [6]. The PERVs were released from porcine cell lines, including pig kidney cell lines PK15 (porcine kidney) and MPK (minipig kidney) and mitogenically activated peripheral blood mononuclear cells (PBMCs) [7–10]. Several strategies have been proposed and could potentially be employed to reduce PERV transmission in xenograft recipients, such as the use of nontransmitter pigs and transgenic pigs expressing siRNAs, as well as the use of restriction factor human APOBEC3G (huA3G), reverse transcriptase inhibitors, viral vaccines, and single-domain antibodies directed against PERV Gag [11–17].

The apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3 (APOBEC3) family consists of seven members (huA3A, huA3B, huA3C, huA3D, huA3F, huA3G and huA3H) in humans. Placental mammals

encode at least one APOBEC3 protein [18]. In pigs, the two APOBEC3 genes encode at least four different mRNAs [12]. The two most potent inhibitors of human immunodeficiency virus (HIV-1) replication are huA3F and huA3G [19]. huA3G binds to RNA, and RNA is brought into the particle by nucleocapsid (NC) domain of Gag. huA3G induces hypermutation of HIV-1 proviruses by editing C to U in the proviral (−) strand during reverse transcription, leading to G-to-A hypermutation. These mutations destabilize and block the functional HIV-1 proviruses, although deaminase-independent mechanisms of inhibition have been reported [20, 21]. In the case of HIV-1, Vif (virion infectivity factor) binds to huA3G in the infected cell and induces its degradation by ubiquitination. Therefore, huA3G inhibits the infectivity of HIV-1 variants lacking a *vif* gene. Wild-type HIV-1 expresses *vif*, which prevents packaging of huA3G into particles [22]. huA3G is capable of inhibiting the replication of a wide variety of retroviruses (HIV, simian immunodeficiency virus [SIV], human T-cell lymphotropic virus [HTLV], murine leukemia virus [MLV], PERV and xenotropic murine leukemia virus-related virus [XMRV]), non-LTR retrotransposons and LTR retrotransposons [23–26]. However, huA3F has been reported to be less inhibitory and less sensitive to Vif than hA3G [27]. huA3G prefers to edit 5′-CC-3′ (where the underline indicates the edited residue) on the proviral DNA (−) strand, while huA3F prefers to edit 5′-TC-3′. Since huA3F is a weak inhibitor of MLV infectivity [28], we were also interested in whether PERV-C, which is most closely related to PERV-A and PERV-B and also closely related to MLV, would be inhibited by huA3F.

Some studies have shown that A3G inhibits PERV-B [12, 13, 15], but one of them [13] did not find any anti-PERV-B activity of poA3Z2-Z3. In this study, VSV-G pseudotyped PERV-C particles were generated in human embryonic kidney (HEK) 293T cells in the presence of APOBEC3 expression plasmids to determine whether the ecotropic PERV-C is affected by APOBEC3 proteins. In addition, 293-PERV-PK-CIRCE clones overexpressing poA3Z2-Z3 were established to determine whether expression of poA3Z2-Z3 would inhibit the transfer of PERV within human cells. By showing that restriction factors can inhibit PERV replication, our study suggests a new approach for minimizing the likelihood of PERV transmission.

Materials and methods

Cell lines

The cell lines 293 human embryonic kidney (ATCC 1573), 293T human embryonic kidney (ATCC CRL-11268), MPK (minipig kidney; ATCC CCL-166), PK-15 pig kidney (ATCC CCL-33), 293-PERV-PK-CIRCE (ECACC 97051411; human 293 cells infected with PK15-derived PERVs), and T cell line H9 (KCLB 30176) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal calf serum (FCS) and 100 U of penicillin and 100 µg of streptomycin per ml.

Cloning of the huA3F, poA3Z2-Z3 and Vif

To amplify human APOBEC3F sequences, total RNA was extracted from the H9 cells, using TRI-Reagent (Molecular Research Center; Cincinnati, OH, USA) according to the manufacturer's instructions. A primer pair was designed to amplify huA3F (GenBank accession number NM_145298). The primers huA3F-HA-for 5′-GGTACCATGAAGCCT CACTTCAGA-3′ (*Kpn*I restriction site is underlined) and huA3F-HA-rev 5′-CTCGAGTTCTCAAGAATCTCCTGC-3′ (*Xho*I restriction site is underlined) were used for RT-PCR. The PCR products of huA3F were ligated into pGEM-T Easy Vector (Promega; Madison, USA). The huA3F-ligated vectors were digested with the restriction enzymes *Kpn*I and *Xho*I. C-terminally HA-tagged huA3F was produced by introducing a *Kpn*I-*Xho*I fragment into the pcDNA3-huA3G-3×HA vector (pcDNA3.1(+)-huA3F-3×HA). Similarly, the poA3Z2-Z3 sequence was amplified, using cDNA generated from PK-15. poA3Z2-Z3-HA-for 5′-GGTACCAT GGATCCTCAGCGCCTG-3′ (*Kpn*I restriction site is underlined) and poA3Z2-Z3-HA-rev 5′-CTCGAGTTTCTT GAGTCACTTCTT-3′ (*Xho*I restriction site is underlined) were used for RT-PCR. C-terminally HA-tagged poA3Z2-Z3 was produced by introducing a *Kpn*I-*Xho*I fragment into pcDNA3-huA3G-3×HA (pcDNA3.1(+)-poA3Z2-Z3-3×HA). Vif DNA was amplified from a pNL4-3 proviral plasmid [29] and inserted into pIRES2-EGFP as *Xho*I-*EcoR*I-digested fragments. vif-for 5′-ACTCGAGATGGAA AACAGATGG-3′ (*Xho*I restriction site is underlined) and vif-rev 5′-TGAATTCCTAGTGTCCATTCAT-3′ (*EcoR*I restriction site is underlined) were used for PCR. Stably huA3G- and poA3Z2-Z3-expressing 293-PERV-PK-CIRCE cell lines were selected using medium containing G418 (1 mg/ml) and APOBEC3-expressing clones were identified by Western blot using an anti-HA antibody.

Western blot assay

APOBEC3 protein expression in transfected cells and its presence in virions in the supernatant were determined by immunoblot analysis. Cell lysates from pcDNA3.1(+)-huA3G-3×HA- and pcDNA3.1(+)-poA3Z2-Z3-3×HA-transfected 293-PERV-PK-CIRCE cells were prepared two days after transfection by lysing the cells in 300 µl of mammalian protein lysis buffer. All samples were denatured at 100 °C for 10 min and then separated by

sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) and then probed with anti-HA antibodies (1:200) (Bethyl, Montgomery, USA) for 1 h or overnight, followed by a 1:5000 dilution of goat anti-rabbit antibody conjugated with horseradish peroxidase (Komabio, Seoul, Korea) for 1 h. The blots were visualized, using 3,3-diaminobenzidine (Bio-Rad, Hercules, CA, USA). Viral lysates from the supernatant of the transfected 293-PERV-PK-CIRCE cells were also tested by Western blot for the expression of APOBEC3.

Virus production and pseudotype assay

The plasmid constructs pCMV-VSV-G, pVPack-PERV-B-GP, pVPack-PERV-C-GP and pCLMFG-*lac*Z (Imgenex Co.; San Diego, USA) were used to construct PERV pseudotype viruses. To generate the PERV-*gag-pol*-expressing plasmid pVPack-PERV-B-GP, *Not*I-*Hpa*I fragments of the amplified PCR products and an *Hpa*I-*Hpa*I fragment from a PERV full-length molecular clone were cloned into pVPack-Eco (Stratagene; La Jolla, USA). To generate the PERV-*gag-pol*-expressing plasmid pVPack-PERV-C-GP, *Cla*I-*Cla*I fragments of the amplified PCR products from cDNA of MPK cell lines were cloned into pVPack-Eco. To investigate the effects of restriction factors, 293T cells were transfected with pCMV-VSV-G (1 μg), pVPack-PERV-B-GP(1 μg), pVPack-PERV-C-GP (1 μg), pCLMFG-*lac*Z (1 μg) and each restriction factor expression plasmid (1 μg), using a PolyFect Transfection Kit (QIAGEN; Valencia, USA). Viral supernatants were collected from each transfection culture and used to infect 293 cells that had been plated in 6-well culture dishes at a density of 1.5×10^5 per well. The cells were infected with 1 ml of virus in the presence of 8 μg polybrene per ml for 3 h, and 2 ml of fresh medium was then added to each well. Two days after infection, the cells were fixed with 0.5 % glutaraldehyde and stained to reveal the presence of β-gal activity. Infectious titers were expressed as the blue CFU count per milliliter of virus supernatant.

Virus-spreading assay by qPCR (quantitative polymerase chain reaction)

The supernatant from restriction-factor-transfected 293-PERV-PK-CIRCE cells was titrated on 293 cells using SYBR Green-based quantitative RT-PCR. The primers PERV-*gag*-for (5′-TGATCTAGTGAGAGAGGCAGAG-3′), PERV-*gag*-rev (5′-CGCACACT GGTCCTTGT-CG-3′), beta-actin-for 5′-ATCATGTTTGAGACCTTCAA-3′ and beta-actin-rev 5′-AGATGGGCACAGTGTGGGT-3′ were used. The number of copies for each gene was quantified from the standard curves. Determination of absolute virion RNA copy numbers was performed using an external homologous standard. The plasmid pBlu-PERV-B harboring the PERV-B *gag*, was digested with *SpeI* and used as the target in an *in vitro* transcription performed with a TranscriptAid T7 High Yield Transcription Kit (Fermentas Inc; Glen Burnie, MD, USA). RNAs were purified, and their concentrations were determined by measuring absorbance at 260 nm. C_T (threshold) values for each dilution (from 10^9 to 10 copies) were measured using real-time RT-PCR to generate standard PERV-B curves. PERV-B copy numbers were normalized to those of beta actin. Real-time RT-PCR was conducted, using an SYBR Premix Ex Taq II Kit (Takara; Shiga, Japan). A Thermal Cycler Dice Real Time System (Takara; Shiga, Japan) was used for thermal cycling and to record fluorescence changes.

Results

Cloning of huA3F, poA3Z2-Z3 and Vif

Molecular cloning of huA3F and poA3Z2-Z3 was performed by reverse transcription PCR, using RNAs extracted from H9 and PK-15 cells. The huA3F and poA3Z2-Z3 proteins were 37 % identical to each other and 49 % and 38 % identical, respectively, to huA3G. C-terminal HA-tagged huA3F and poA3Z2-Z3 were produced (Fig. 1A) when the same amount of expression vector was used for transfection, and huA3F and poA3Z2-Z3 proteins were expressed at equivalent levels (Fig. 1B). The APOBEC3s, with a molecular mass of 46 kDa (huA3G), 45 kDa (huA3F) and 51 kDa (poA3Z2-Z3), were detected in the lysate of transfected 293T cells. To compare the ability of the HIV-1 Vif protein to reduce the levels of huA3G, huA3F and poA3Z2-Z3 proteins, *vif* was amplified from pNL4-3 proviral plasmid and inserted into pIRES2-EGFP.

Comparison of antiviral activity of huA3G, huA3F and poA3Z2-Z3

To test the susceptibility of PERV-B and PERV-C to restriction by APOBEC3 proteins, we produced VSV-pseudotyped PERVs by transfection of 293T cells with plasmids expressing PERV Gag-Pol, VSV-G envelope, and pCLMFG-*lac*Z in the presence or absence of APOBEC3s. Pseudotype virus titers were determined by infection of 293 cells. The antiviral activity of porcine A3F against the PERV-B pseudotype was slightly weaker than that of huA3G. In contrast, a porcine A3F against the PERV-C pseudotype was more potent than huA3G.

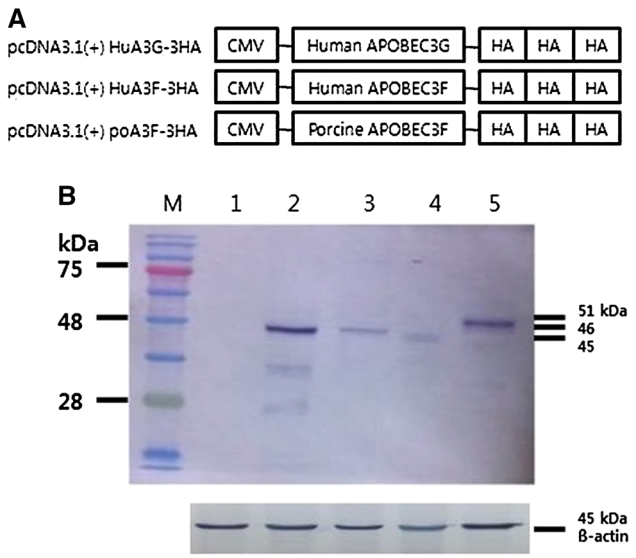

Fig. 1 Cloning and expression of various APOBEC3 proteins in 293T cells. (A) Schematic representation of APOBEC3 plasmid constructs. huA3F and poA3Z2-Z3 were amplified and were cloned into the pcDNA3.1(+)-huA3G-3×HA plasmids. (B) 293T cells were transfected with huA3G (1 μg and 0.5 μg), huA3F (0.5 μg) and poA3Z2-Z3 (0.5 μg) expression vector, and the protein that was expressed was detected by Western blot, probed with anti-HA antibody. Lane M, solgent™ triple color protein marker; lane 1, 293T cells transfected with empty vector DNA; lanes 2-5, huA3G (1 μg), huA3G (0.5 μg), huA3F (0.5 μg) and poA3Z2-Z3 (0.5 μg). Equal loading was controlled by monitoring β-actin. poA3F is poA3Z2-Z3

Several studies have shown a dose-dependent relationship between huA3G levels and virus infectivity [30–32]. In agreement with previous results, the infectivity of PERV decreased as intracellular APOBEC3 protein levels increased. These APOBEC3 proteins restricted PERV-B and PERV-C infection in a dose-dependent manner (Fig. 2A, and B)

Relative sensitivity of huA3G, huA3F and poA3Z2-Z3 proteins to Vif

HIV-1 accessory protein Vif (virion infectivity factor) forms a complex with human APOBEC3G that prevents its encapsidation in virions. APOBEC3G proteins are degraded via E3-mediated ubiquitination. Previous reports have suggested that the Vif proteins are less effective at counteracting the antiviral effect of huA3F than that of huA3G [27]. To examine the relative sensitivity of APOBEC3 proteins to Vif, we compared the expression levels of huA3G, huA3F and poA3Z2-Z3 proteins in transfected 293T cells. As can be seen in Fig. 3, the Vif proteins reduced the level of APOBEC3 proteins in transfected cells. Vif proteins eliminated huA3G and huA3F proteins from cells with greater potency than they did poA3Z2-Z3.

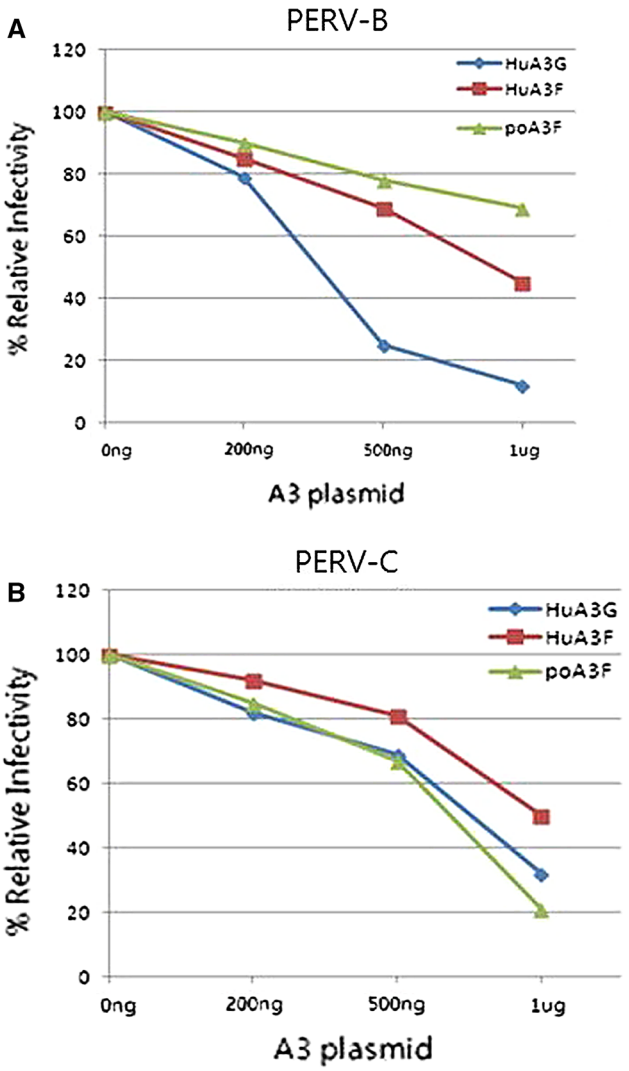

Fig. 2 Comparison of the effects of huA3G, huA3F and poA3Z2-Z3 on antiviral activity. 293T cells were co-transfected with pCMV-VSV-G, pVPack-PERV-B-GP, pVPack-PERV-C-GP, pCLMFG-*lac*Z and increasing amounts of APOBEC3 expression plasmids (0.2-1 μg). PERV-B (A) and PERV-C (B) pseudotypes were inhibited by APOBEC3 proteins in a dose-dependent manner. poA3F is poA3Z2-Z3

Virus-spreading assay by qPCR

A qPCR assay was used to investigate whether the expression level of huA3G and poA3Z2-Z3 correlated with virus infectivity. The huA3G and poA3Z2-Z3 stable cell lines were constructed by transfecting 293-PERV-PK-CIRCE cells with pcDNA3.1(+)-huA3G-3×HA and pcDNA3.1(+)-poA3Z2-Z3-3×HA. Stable expression of huA3G and poA3Z2-Z3 were compared to 293-PERV-PK-CIRCE cells transiently transfected with increasing amounts of pcDNA3.1(+)-huA3G-3×HA and pcDNA3.1(+)-poA3Z2-Z3-3×HA. The results showed that the expression level of APOBEC3 proteins in transiently transfected

293 cells was higher than that in the stable cells. However, we observed efficient packaging of huA3G and poA3Z2-Z3 from stable APOBEC3-expressing cell lines (Fig. 4A and Fig. 5A). The infectivity of PERV produced from stable APOBEC3-expressing cell lines was much higher than PERV produced from transiently transfected cells (Fig. 4B and Fig. 5B). This suggests that encapsidation can be limited by severe overexpression of the APOBEC3 proteins in transiently transfected cells.

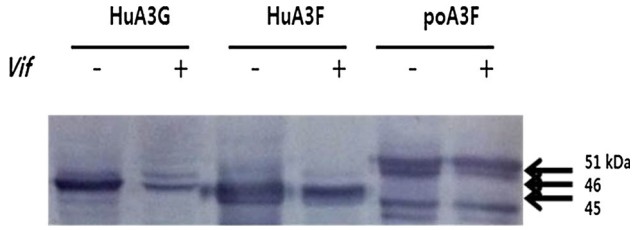

Fig. 3 Downregulation of APOBEC3 protein expression by HIV-1 *vif*. 293T cells were transfected with PERV-B vector expression plasmids and 2 μg of APOBEC3 expression plasmids in the absence or presence of 1 μg of each Vif expression plasmid. Lysates of transfected cells were analyzed by Western blotting to determine APOBEC3 expression levels

Discussion

A recent screening for PERV-C-free animals among non-transgenic and multitransgenic animals demonstrated that 92 % of animals were PERV-C positive [33, 34]. Although the prevalence and distribution of PERV-C seem to depend on the pig strain, PERV-C is widely distributed. Moreover, two defective retroviral sequences copackaged into PERV particles can participate in recombination events, leading to a replication-competent virus. In addition to selection of PERV-C-negative animals, inhibition of PERV replication by intracellular restriction factor is an important novel approach to minimize or avoid transmission of PERV.

It has been reported that the effects of mouse APOBEC3 on Moloney MLV (MoMLV) are different from its effects on many MLVs [26]. MoMLV is partially resistant to inactivation by mouse APOBEC3 and does not involve G→A hypermutation [35]. It appears that differences between Gag proteins of MoMLV and those of other MLVs might be responsible for the difference in sensitivity to mouse APOBEC3-induced hypermutation [24]. However, recent reports have demonstrated that a chimeric MLV in which the *gag* gene of ecotropic MoMLV was replaced by that of polytropic endogenous virus, showed the same

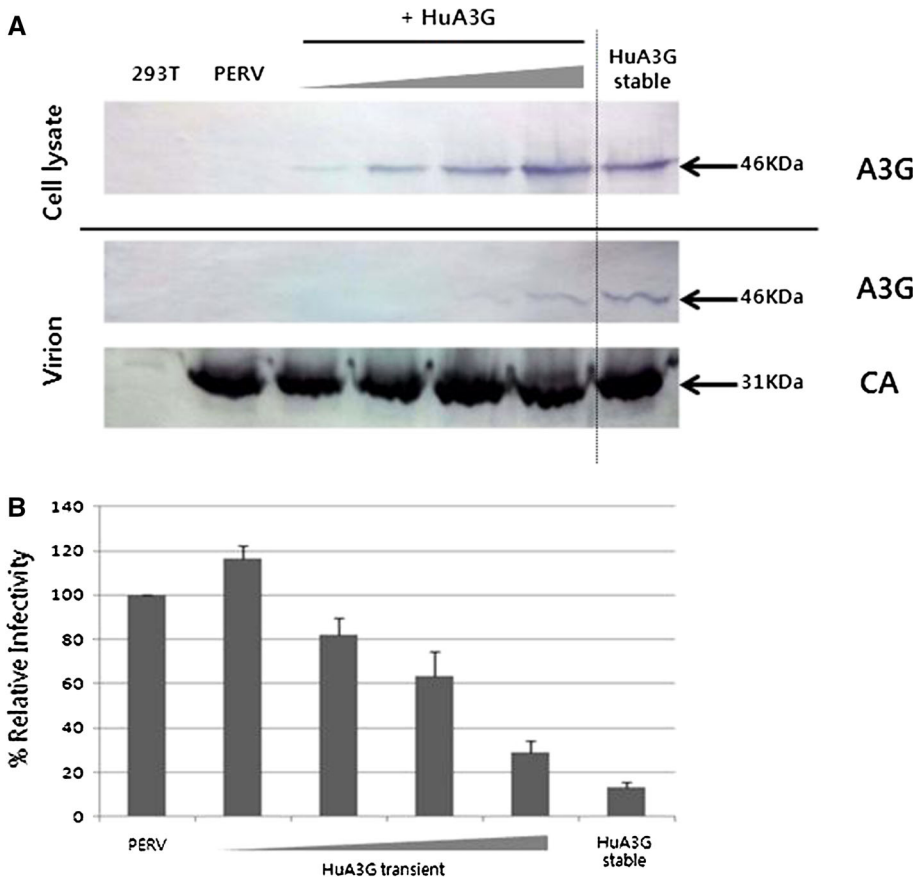

Fig. 4 Comparison of the effect of transiently and stably expressed huA3G on PERV infectivity. (A) 293-PERV-PK-CIRCE cells were transfected with various amounts of huA3G expression plasmids (0.5 μg, 1 μg, 3 μg, and 5 μg). Cell and viral lysates were analyzed by Western blot using anti-HA antibodies and antiserum against PERV capsid (CA) to measure APOBEC3 expression. (B) Viral supernatants were harvested 48 h post-transfection and used to infect 293 cells. At 48 h postinfection, viral RNA extracted from infected 293 cells was analyzed by real-time RT-PCR, based on the PERV *gag* gene. PERV copy numbers were normalized to those of β-actin. The infectivity of PERV produced in empty-vector-transfected cells was set to 100 %. Error bars represent the standard deviations of three independent experiments

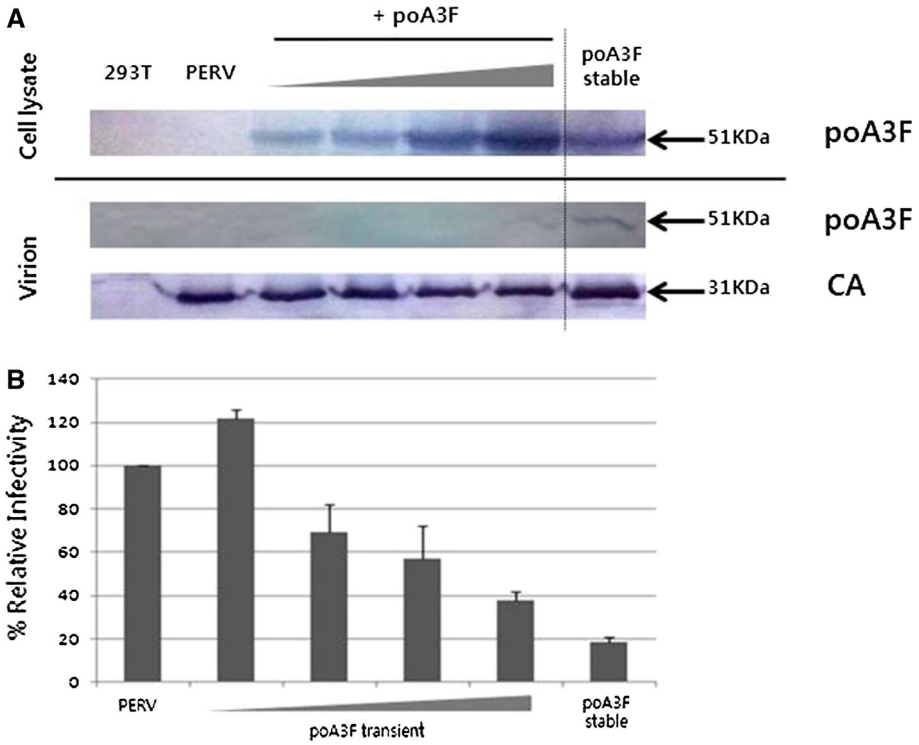

Fig. 5 Comparison of transiently and stably expressed poA3Z2-Z3 on PERV infectivity. (A) poA3Z2-Z3-containing supernatants were collected after transfection of 293-PERV-PK-CIRCE cells with different amounts of poA3Z2-Z3 expression plasmids (0.5 μg, 1 μg, 3 μg, and 5 μg) or a control transfection without plasmid. Cell and viral lysates were analyzed by Western blot using anti-HA antibodies and antiserum against PERV capsid (CA) to measure APOBEC3 expression. (B) Virus-containing supernatants were harvested 48 h post-transfection and used to infect 293 cells. The relative infectivity of PERV was determined as described in Materials and methods. Error bars represent the standard deviations of three independent experiments. poA3F is poA3Z2-Z3

response to mouse APOBEC3 as MoMLV [26]. Here, we were interested in whether the effects of APOBEC3s on polytropic PERV-B are different from those of ecotropic PERV-C.

Previous studies have demonstrated that PERV-B is inhibited by various APOBEC3 proteins, but little is known about ecotropic PERV-C [12, 15]. We asked whether PERV-C would package various APOBEC3 proteins into virions, and the susceptibility of PERV-C to restriction by various APOBEC3 proteins was tested. As shown in Fig. 2B, PERV-C appears to be more sensitive to poA3Z2-Z3 than to huA3G. The reduction of PERV-C infectivity by poA3Z2-Z3 was more efficient than that seen for PERV-B. This reduction of PERV-C infectivity may be correlated with the differential ability of poA3Z2-Z3 proteins to bind PERV Gag. The amino acid sequence identity between PERV-B (accession number AJ133816) and PERV-C (accession number AF038600) is 94.6 % for *gag*. It seems possible that amino acid sequence differences may be responsible for the difference in the sensitivity of PERVs to poA3Z2-Z3. The response of PERV-C to huA3F was qualitatively indistinguishable from that of PERV-B. In addition, increasing amounts of the APOBEC3 plasmids in the transfection caused a progressive reduction of PERV infectivity (Fig. 2A and B).

Vif is a 190- to 240-amino-acid protein that is encoded by lentiviruses. Most lentiviruses counteract APOBEC3 protein activity by expressing Vif, which prevents its incorporation into virions [25]. HIV-1 Vif does not form a complex with mouse APOBEC3 and does not prevent its encapsidation [36]. PERV does not encode a Vif protein. We compared the ability of Vif to reduce the expression levels of APOBEC3 proteins. The effects of the Vif proteins on APOBEC3 protein levels were different, but Vif eliminated APOBEC3 proteins (Fig. 3). Vif proteins appeared to be more potent and specific inhibitors of huA3G than of poA3Z2-Z3. As described previously, HIV-1Vif neutralized huA3G and huA3F. However, poA3Z2-Z3 was resistant to Vif.

Previous studies showed that the PK-15 clones overexpressing poA3Z2-Z3 did not significantly interfere with PERV transmission [15], but other groups suggested that poA3Z2-Z3 could inhibit PERV replication [12]. The major difference was in the experimental system used. Some groups used porcine kidney cell line (PK-15) clones stably expressing poA3Z2-Z3, while other groups used stable poA3Z2-Z3-expressing human 293T cell lines and a

plasmid encoding a replication-competent PERV-B clone to generate virus particles. To determine whether this difference could be responsible for the contradictory results, we generated 293-PERV-PK-CIRCE cells stably expressing huA3G and poA3Z2-Z3. It has been reported that stably expressed huA3F has negligible antiviral activity [37]. We compared antiviral effects between transiently transfected cells and stably transfected cells. As shown in Fig. 5, poA3Z2-Z3 from stable 293-PERV-PK-CIRCE cells is a strong inhibitor of PERV infectivity. In agreement with previous results [12], poA3Z2-Z3 was able to restrict PERV replication. Further studies are required to determine whether PERV from PK-15 cells develops adaptive mutations that can overcome the poA3Z2-Z3 restriction. The amount of virus-associated poA3Z2-Z3 in transient expression appeared to be directly proportional to the intracellular protein levels. Although transient expression of poA3Z2-Z3 can lead to severe overexpression of the protein, the efficiency of poA3Z2-Z3 packaging is not as good as poA3Z2-Z3 packaging from stable cells.

In conclusion, we have demonstrated that ecotropic PERV-C virions can package huA3G, huA3F and poA3Z2-Z3. These APOBEC3 proteins restricted PERV-C infection in a dose-dependent manner, and PERV-C was significantly restricted by poA3Z2-Z3. PERVs produced from stably poA3Z2-Z3-expressing cells contain significantly more poA3Z2-Z3 than viruses derived from transiently poA3Z2-Z3-expressing cells. Further studies are needed to elucidate how PERVs can avoid restriction by poA3Z2-Z3 expressed in their normal host species.

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References

1. Boneva RS, Folks TM, Chapman LE (2001) Infectious disease issues in xenotransplantation. Clin Microbiol Rev 14:1–14
2. Magre S, Takeuchi Y, Bartosch B (2003) Xenotransplantation and pig endogenous retroviruses. Rev Med Virol 13:311–329
3. Patience C, Takeuchi Y, Weiss RA (1997) Infection of human cells by an endogenous retrovirus of pigs. Nat Med 3:282–286
4. Takeuchi Y, Patience C, Magre S, Weiss RA, Banerjee PT, Le Tissier P, Stoye JP (1998) Host range and interference studies of three classes of pig endogenous retrovirus. J Virol 72:9986–9991
5. Bartosch B, Stefanidis D, Myers R, Weiss R, Patience C, Takeuchi Y (2004) Evidence and consequence of porcine endogenous retrovirus recombination. J Virol 78:13880–13890
6. Harrison I, Takeuchi Y, Bartosch B, Stoye JP (2004) Determinants of high titer in recombinant porcine endogenous retroviruses. J Virol 78:13871–13879
7. Lieber MM, Sherr CJ, Benveniste RE, Todaro GJ (1975) Biologic and immunologic properties of porcine type C viruses. Virology 66:616–619
8. Patience C, Takeuchi Y, Weiss RA (1997) Infection of human cells by an endogenous retrovirus of pigs. Nat Med 3:282–286
9. Todaro GJ, Benveniste RE, Lieber MM, Sherr CJ (1974) Characterization of a type C virus released from the porcine cell line PK(15). Virology 58:65–74
10. Wilson CA, Wong S, Muller J, Davidson CE, Rose TM, Burd P (1998) Type C retrovirus released from porcine primary peripheral blood mononuclear cells infects human cells. J Virol 72:3082–3087
11. Dieckhoff B, Karlas A, Hofmann A, Kues WA, Petersen B, Pfeifer A, Niemann HR, Denner KJ (2007) Inhibition of porcine endogenous retroviruses (PERVs) in primary porcine cells by RNA interference using lentiviral vectors. Arch Virol 152:629–634
12. Dörrschuck E, Fischer N, Bravo IG, Hanschmann KM, Kuiper H, Spötter A, Möller R, Cichutek K, Münk C, Tönjes RR (2011) Restriction of porcine endogenous retrovirus by porcine APOBEC3 cytidine deaminases. J Virol 85:3842–3857
13. Dörrschuck E, Münk C, Tönjes RR (2008) APOBEC3 proteins and porcine endogenous retroviruses. Transpl Proc 40:959–961
14. Fiebig U, Stephan O, Kurth R, Denner J (2003) Neutralizing antibodies against conserved domains of p15E of porcine endogenous retroviruses: basis for a vaccine for xenotransplantation? Virology 307:406–413
15. Jónsson SR, LaRue RS, Stenglein MD, Fahrenkrug SC, Andrésdóttir V, Harris RS (2007) The restriction of zoonotic PERV transmission by human APOBEC3G. PLoS One 12:e893
16. Mattiuzzo G, Ivol S, Takeuchi Y (2010) Regulation of porcine endogenous retrovirus release by porcine and human tetherins. J Virol 84:2618–2622
17. Qari SH, Magre S, García-Lerma JG, Hussain AI, Takeuchi Y, Patience C, Weiss RA, Heneine W (2001) Susceptibility of the porcine endogenous retrovirus to reverse transcriptase and protease inhibitors. J Virol 75:1048–1053
18. Harris RS, Liddament MT (2004) Retroviral restriction by APOBEC proteins. Nature 4:868–877
19. Mbisa JL, Bu W, Pathak VK (2010) APOBEC3F and APOBEC3G inhibit HIV-1 DNA integration by different mechanisms. J Virol 84:5250–5259
20. Aguiar RS, Peterlin BM (2008) APOBEC3 proteins and reverse transcription. Virus Res 134:74–85
21. Holmes RK, Malim MH, Bishop KN (2007) APOBEC-mediated viral restriction: not simply editing? Trends Biochem Sci 32:118–128
22. Goila-Gaur R, Strebel K (2008) HIV-1 Vif, APOBEC, and intrinsic immunity. Retrovirology 5:51
23. Bogerd HP, Zhang F, Bieniasz PD, Cullen BR (2011) Human APOBEC3 proteins can inhibit xenotropic murine leukemia virus-related virus infectivity. Virology 410:234–239
24. Langlois MA, Kemmerich K, Rada C, Neuberger MS (2009) The AKV murine leukemia virus is restricted and hypermutated by mouse APOBEC3. J Virol 83:11550–11559
25. Sadler HA, Stenglein MD, Harris RS, Mansky LM (2010) APOBEC3G contributes to HIV-1 variation through sublethal mutagenesis. J Virol 84:7396–7404
26. Sanchez-Martinez S, Aloia AL, Harvin D, Mirro J, Gorelick RJ, Jern P, Coffin JM, Rein A (2012) Studies on the restriction of murine leukemia viruses by mouse APOBEC3. PLoS One 7:e38190
27. Zennou V, Bieniasz PD (2006) Comparative analysis of the antiretroviral activity of APOBEC3G and APOBEC3F from primates. Virology 349:31–40
28. Bogerd HP, Zhang F, Bieniasz PD, Cullen BR (2011) Human APOBEC3 proteins can inhibit xenotropic murine leukemia virus-related virus infectivity. Virology 410:234–239

29. Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, Rabson A, Martin MA (1986) Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J Virol 59:284–291
30. Kao S, Khan MA, Miyagi E, Plishka R, Buckler-White A, Strebel K (2003) The human immunodeficiency virus type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity. J Virol 77:11398–11407
31. Miyagi E, Opi S, Takeuchi H, Khan M, Goila-Gaur R, Kao S, Strebel K (2007) Enzymatically active APOBEC3G is required for efficient inhibition of human immunodeficiency virus type 1. J Virol 81:13346–13353
32. Xu H, Chertova E, Chen J, Ott DE, Roser JD, Hu WS, Pathak VK (2007) Stoichiometry of the antiviral protein APOBEC3G in HIV-1 virions. Virology 360:247–256
33. Dieckhoff B, Kessler B, Jobst D, Kues W, Petersen B, Pfeifer A, Kurth R, Niemann H, Wolf E, Denner J (2009) Distribution and expression of porcine endogenous retroviruses in multi-transgenic pigs generated for xenotransplantation. Xenotransplantation 16:64–73
34. Kaulitz D, Mihica D, Dorna J, Costa MR, Petersen B, Niemann H, Tönjes RR, Denner J (2011) Development of sensitive methods for detection of porcine endogenous retrovirus-C (PERV-C) in the genome of pigs. J Virol Methods 175:60–65
35. Browne EP, Littman DR (2008) Species-specific restriction of apobec3-mediated hypermutation. J Virol 82:1305–1313
36. Mariani R, Chen D, Schröfelbauer B, Navarro F, König R, Bollman B, Münk C, Nymark-McMahon H, Landau NR (2003) Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 114:21–31
37. Miyagi E, Brown CR, Opi S, Khan M, Goila-Gaur R, Kao S, Walker RC Jr, Hirsch V, Strebel K (2010) Stably expressed APOBEC3F has negligible antiviral activity. J Virol 84:11067–11075