Evaluation of the Potential Risk of Porcine Endogenous Retrovirus (PERV) Infection in Nude Mice

Bae, Eun Hye and Yong-Tae Jung*

Department of Microbiology and Institute of Basic Science, Dankook University, Cheonan 330-714, Korea

Received: December 10, 2010 / Revised: January 4, 2011 / Accepted: January 5, 2011

Nude mice (BALB/c) were grafted with human 293 cells and PERV (porcine endogenous retrovirus)-IRES-EGFP (a packageable retroviral vector plasmid containing an internal ribosome entry site-enhanced green fluorescent protein)-producing pig PK15 cells in order to determine whether the pig cells could transmit PERV-IRES-EGFP to mice and human 293 cells in vivo. None of the transplanted human 293 cell lines were infected by PERV, but PCR analysis identified PERV-B provirus integration into both the heart and salivary gland of the inoculated nude mice. Our data indicate that hearts and salivary glands can be used to identify PERV-B receptors.

Keywords: Animal model, nude mice, porcine endogenous retroviruses, replication-defective retroviral vector, xenotransplantation

Xenotransplantation, the transplantation of pig cells, tissues, and organs into human patients, is used to relieve the shortage of human organs. However, infectious pig pathogens are a major concern when porcine tissues are used for transplantation [2, 3, 18]. Porcine endogenous retroviruses (PERVs) are of particular concern because they are capable of infecting human cells in vitro [7, 10]. Two classes of infectious human-tropic replication-competent (HTRC) PERVs (polytropic PERV-A and PERV-B) and one class of ecotropic PERV-C have been identified [1, 15, 22, 24]. The PERVs were released from porcine cell lines including pig kidney cell lines PK15 [23], MPK, and mitogenically activated peripheral blood mononuclear cells (PBMC). Researchers have tried to establish small-animal models to better evaluate the risk of PERV in vivo [16]. Several investigators have attempted to infect baboons, rhesus macaque, bonnet macaques, and pig-tailed monkeys with PERV to establish non-human primate models to study PERV replication. However, attempts to develop a large-animal model have been unsuccessful [13, 17, 20]. Transmission of PERVs in vivo has only been described in severe combined immunodeficient (SCID) and nude mice inoculated with PERV-producing cells [4, 26, 27]. However, it was previously reported that PERV transmissions to SCID and nude mice, in vivo, might be due to microchimerism or pseudotyping with murine leukemia viruses [8, 9, 11, 25]. In this study, the in vivo infectivity of PERVs was evaluated by establishing a nude mouse model system, which was designed for the easy and convenient detection of microchimerisms. We have herein used a conventional defective retroviral vector system; this helper-dependent system consists of a defective retrovirus containing the EGFP reporter gene [19]. Infectious particles carrying the EGFP can only be generated in cells coexpressing the defective vector and the wild-type virus genomes, and therefore this system can be used to study the risk of PERV in vivo infection.

293-PERV-PK-CIRCE (ECACC 97051411), 293 human embryonic kidney (ATCC 1573), and PK15 pig kidney (ATCC CCL-33) cells were maintained in DMEM supplemented with 10% fetal calf serum, 100 U penicillin/ml, and 100 µg streptomycin/ml. To generate a PERV-B env expression plasmid, pRES2-EGFP-PERV-B, the SacI–EcoRI fragment of pBluescript-PERV-B was inserted directly into the same sites of pRES2-EGFP [14, 22]. To generate a retroviral vector containing an enhanced GFP (EGFP) and PERV-B env, the NotI–NotI fragment of pRES2-EGFP-PERV-B was excised and ligated into the pLPCX retroviral vector (pLPCX-PERV-B-IRES2-EGFP). To test whether RNA from the vector can be packaged into replication incompetent retroviral particles, pLPCX-PERV-B-IRES2-EGFP was introduced into 293-PERV-PK-CIRCE cells. Virus-containing

*Corresponding author
Phone: +82-41-550-3453; Fax: +82-41-559-7857; E-mail: yjung@dankook.ac.kr
supernatant was used to infect fresh 293 cells. Three days post-infection, the numbers of EGFP-expressing foci were determined using fluorescence microscopy. The pLPCX-PERV-B-IRE52-EGFP was also introduced into PERV-producing PK15 cells, and individual puromycin-resistant cell clones were selected. Six-week-old female BALB/c nude mice (n=8; ORIENT BIO Inc., Korea) were injected subcutaneously with $1 \times 10^7$ PERV-B-IRES-EGFP-producing pig PK15 cells in the left flank and $1 \times 10^7$ human 293 cells in the right flank. Tumors appeared at the inoculation site 3 weeks after injection, and metastases were not observed. Tumors were removed after 3 weeks of propagation, and EGFP-expressing cells were analyzed by fluorescence microscopy. DNA was extracted from porcine and human cells and from mice organs, including the liver, kidney, lungs, heart, salivary gland, and spleen, using the RBC genomic DNA extraction kit (RBC Bioscience, Taiwan) according to the manufacturer’s instructions. The primers used for PCR amplification of PERV sequences allow for the detection of gag, pol, and env. The primers PMTF/PMTR were used to amplify pig mtDNA cytochrome oxidase subunit II (COII) for the detection of pig cellular DNA in mouse DNA samples (Table 1).

We constructed a defective retrovirus containing the EGFP reporter gene, and then transfected this construct into PERV-producing 293-PERV-PK-CIRCE cells (Fig. 1A). To investigate whether replication-competent PERV can pseudotype MLV vector transcripts, the supernatants of transfected cells were used to infect 293 cells. Even though the vector genome was not efficiently packaged, EGFP-expressing cells were detected by fluorescence microscopy (Fig. 1B). To determine whether pig cells could transmit PERV to mouse and human cells in vivo, we used a helper-dependent vector system. If PERV is able to infect human 293 cells in vivo, EGFP-transduced cells will have been observed in the human 293 tumor cells. The expression vector pLPCX containing the PERV-B env-EGFP was established and transfected into PERV-producing PK15 cells. After selecting PERV-B-EGFP-PK15 cells with puromycin, transgene expression was identified by fluorescence microscopy. Porcine PK15 tumors were generated by injecting these stably transfected cells into nude mice. After 3 weeks, the tumors were removed, digested with 1 ml of 0.25% trypsin in PBS/5 mg of tissue, and then cultivated in DMEM, in vitro, for 5 days. We detected bright EGFP-expressing PK15 tumor cells (Fig. 2A). These data suggest that EGFP-expressing PK15 tumor cells can be used to enable PERV transmission via cell-to-cell contact. To detect the transmission of PERV from EGFP-expressing PK15 tumor cells to human 293 tumor cells, human 293 tumor cell suspensions were cultivated in DMEM. As shown in Fig. 2B, we could not detect any fluorescence signal. In addition, no PCR products were obtained with the PERV primers on human 293 cells from nude mice (Table 2). This result suggests that the release of PERV from PK15 cells is not capable of infecting human 293 cells in vivo. It also suggests that the xenotropic murine leukemia virus (X-MLV) might not pseudotype PERV in our model. To analyze the integration of proviral

### Table 1. The primers used for polymerase chain reaction analysis of PERV sequences.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’→3’)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERV-B envF</td>
<td>5’-AATTCTCCTTTTGTAATTCGGGCC-3’</td>
<td>270</td>
</tr>
<tr>
<td>PERV-B envR</td>
<td>5’-CCAGTACCTTTACGGGCTCCCACTG-3’</td>
<td>272</td>
</tr>
<tr>
<td>PERV-gagF</td>
<td>5’-TGATCTAGTGGAGGCAAGGAGAG-3’</td>
<td>272</td>
</tr>
<tr>
<td>PERV-gagR</td>
<td>5’-CGCACACTGTCCTTTGTGC-3’</td>
<td>227</td>
</tr>
<tr>
<td>PERV-polF</td>
<td>5’-TCTCCCCAAAGTGACCTGAT-3’</td>
<td>255</td>
</tr>
<tr>
<td>PERV-polR</td>
<td>5’-ACTAGGATGCCTGTTGAATT-3’</td>
<td>255</td>
</tr>
<tr>
<td>PMTF</td>
<td>5’-TTTAACGTTAGGCGTGGATTAAT-3’</td>
<td>255</td>
</tr>
<tr>
<td>PMTR</td>
<td>5’-TTTAACGTTAGGCGTGGATTAAT-3’</td>
<td>255</td>
</tr>
</tbody>
</table>
DNA into the genome of nude mice, DNA was extracted from different mouse organs. The infection of these tissues was analyzed by conventional PCR for the \textit{gag}, \textit{pol}, and \textit{env} genes of PERV-B (Fig. 3). Some mouse samples (hearts and salivary glands) were seen to be positive for PERV \textit{gag}, \textit{pol}, and \textit{env}, but no PCR products were obtained using the \textit{mt COII} primers on nude mice tissues (Table 2). These data indicate that PERV can infect some mouse cells, but cannot infect human 293 cells \textit{in vivo}.

Infection assays using a pseudotyped MLV that harbors the retroviral MFGnlsLacZ vector and different PERV envelopes indicated that PERV-A, -B, and -C each use different receptors [24]. Recently, two genes encoding functional human PERV-A receptors were isolated [6, 12]. Previous attempts to infect cell lines and primary cells from mice \textit{in vitro} were not successful because mouse cells have a nonfunctional receptor for PERV-A. However, the receptor for PERV-B is still unknown. It is possible that a susceptible cell lineage not yet tested \textit{in vitro} might be present in these nude mice, so different organs from nude mice were PCR analyzed using PERV-specific primers (Fig. 3). Among the mouse tissues tested in PK15 and 293 grafted nude mice, 5/8 (62.5\%) samples from hearts and salivary glands were noted as being positive for PERV-B, but negative for pig \textit{mtDNA} cytochrome oxidase subunit II (\textit{COII}) (Table 2). These results suggest that PERV infection occurred in these tissues, but porcine–mouse microchimerism did not. Hearts and salivary glands can be used to identify PERV-B receptors for further experimentation.

**Acknowledgment**

The present research was funded by a research fund of Dankook University in 2010.

**REFERENCES**


