Promoter activity analysis and methylation characterization of LTR elements of PERVs in NIH miniature pig

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The potential risk of porcine endogenous retrovirus (PERV) transmission is an important issue in xenotransplantation (pig-to-human transplantation). Long terminal repeats (LTRs) in PERV elements show promoter activity that could affect neighboring functional genes. The methylation status and promoter activities of 3 LTR structures (PERV-LTR1, LTR2, and LTR3 elements) belonging to the PERV-A family were examined using luciferase reporter genes in human liver cell lines (HepG2 and Hep3B). The PERV LTR3 element exhibited hypomethylation and stronger promoter activity than the other LTR elements in human liver cells. We also performed comparative sequences analysis of the PERV LTR elements by using bioinformatics tools. Our findings showed that several transcription factors such as Nkx2-2 and Elk-1 positively influenced the high transcriptional activity of the PERV LTR3 element.

Key words: long terminal repeat (LTR), methylation, porcine endogenous retrovirus (PERV), promoter activity, transcription factor-binding sites

INTRODUCTION

Recently published studies on xenotransplantation involving transgenic pigs have focused on the prevention of cell-mediated rejection and the inhibition of porcine endogenous retrovirus (PERV) transmission to recipients. PERVs are considered to be a major risk factor associated with xenotransplantation (Patience et al., 1997). PERVs are integrated into all pig genomes, and some PERVs are transcribed and translated (Denner, 2008b). Consequently, some viral proteins are expressed and viral particles are also released in pig cells (Denner, 2008b). These proteins may constitute a potentially high risk for xenotransplantation that involves the use of pig cells or organs. In pig genomes, more than 50 proviral copies of PERVs exist, depending on the pig breed and subtype (Patience et al., 2001). The expression and release of these PERVs occurs in normal pig cells and tumor cell lines (Boneva et al., 2001).

PERVs belong to the genus Gammaretrovirus and, in particular, are related to the feline leukaemia virus, murine leukaemia virus, gibbon ape leukaemia virus, and koala retrovirus, which induce leukaemia and immunodeficiency in infected hosts (Denner, 2007, 2008a). PERVs are classified into 2 groups of viruses: betaretroviruses and gammaretroviruses. Gammaretroviruses consist of 10 families (Klymiuk et al., 2002). Among the subfamilies of gammaretroviruses, PERVs constitute the only subfamily of replication-competent proviruses. Long terminal repeat (LTR) retrotransposons, including PERVs, have been reported with respect to horizontal transfer. Horizontal transfer is defined as the interspecies transfer of a gene or other DNA elements (Flavell, 1999; Jordan et al., 1999). In fact, horizontal transfer of the copia LTR retrotransposon from Drosophila melanogaster to D. willistoni has been previously reported (Jordan et al., 1999). This phenomenon is useful for mobile elements.
but does not allow them to escape their antagonistic host environment (Flavell, 1999). One of the possible mechanisms underlying horizontal transfer involves the use of poxviruses as vectors during evolution (Piskurek and Okada, 2007). PERVs could also transfer to the human genome from the pig genome by employing horizontal transfer. Therefore, the risk of transfer of PERVs has been raised consistently in discussions that concern pig-to-human xenotransplantation.

Intact PERV proviruses harbor the structural genes gag, pol, and env and are flanked by LTRs at both ends (Akiyoshi et al., 1998; Le Tissier et al., 1997). Three distinct PERV families (PERV-A, PERV-B, and PERV-C), which are mammalian type C retroviruses, have been identified according to their envelope protein sequences (Ericsson et al., 2001; Mang et al., 2001; Patience et al., 2001). Two other ORFs, gag and pol, are highly homologous amongst all types of PERVs. Among these subtypes, PERV-A and B infect human cells and are present in the genome of every pig (Martin et al., 1998; Wilson et al., 1998). However, PERV-C infects only pig cells and is not ubiquitously expressed in pigs (Takeuchi et al., 1998). Recombinant PERV-AC has also been reported in various studies, but these recombinant PERVs-AC have been detected only in normal tissues (Bittmann et al., 2012). However, recombinant PERVs could infect and adapt to human cells and thus represent a potential high-risk factor in pig-to-human xenotransplantation (Wood et al., 2004; Karlas et al., 2010). Moreover, completely intact PERV proviruses have been identified, and their replication and infection potential have been examined using human and non-human primate cells (Blusch et al., 2000; Czauderna et al., 2000). Recently, PERVs have been identified in a variety of other pig breeds, including Large White, Westran, Duroc, Landrace, Chinese, and wild pigs (Edamura et al., 2004; Lee et al., 2002; Li et al., 2004; Mang et al., 2001; Niebert et al., 2002); almost 50 copies of replication-competent PERVs are known to exist in pig genomes (Patience et al., 2001).

The LTR is composed of the regions U3, R, and U5, which are known to be important sequences for regulating the transcription of PERV elements (Akiyoshi et al., 1998). Thus, different types of LTR sequences significantly affect the replication properties of PERVs. Generally, the R and U5 regions are conserved sequences. PERV-A and B proviruses contain distinct 39-bp repeats in the U3 regions, which enable PERVs to maintain a high level of transcription (Schep et al., 2001). Thus, mutations such as deletions, insertions, and nucleotide substitutions could transmute the transcriptional activity of a PERV element. PERV LTR elements have been found to harbor potential hormone-responsive sequences (Quinn and Langford, 2001), and the 39-bp repeats in particular, which harbor the transcription factor-binding sites of NF-Y. These transcription factor-binding sites have also been identified as being major key elements that control PERV transcription activities (Quinn and Langford, 2001). Structure analysis of PERV LTR elements has been described in several reports. Generally, the subtypes of PERV LTR elements that resemble PERV-A and -B LTR elements exhibit strong promoter activities regardless of the breed of pig that they inhabit (Park et al., 2010; Ha et al., 2007; Huh et al., 2009; Yu et al., 2012; Jung et al., 2013). However, each PERV LTR element has a different structure according to the breed of pig (Jung et al., 2013; Huh et al., 2009; Ha et al., 2007); of these, only 39-bp tandem repeats constitute a ubiquitous component (Lee et al., 2012; Yu et al., 2012; Jung et al., 2013). Furthermore, transcription factor-binding sites also influence the promoter activities of PERV LTR elements. By using deletion mutant constructs, the cis-elements of the DNA sequences of PERV LTR elements in the U3 region were also found to affect the promoter activities of PERV LTR elements (Ha et al., 2007; Jung et al., 2013). Regulation of the transcriptional activity of PERV LTR elements can be altered by DNA methylation and histone modification. By using M.SssI and garecinol, DNA methylation patterns can be used to regulate the promoter activity of PERV LTR elements (Park et al., 2010). Therefore, PERV LTR elements may control the expression of PERVs as regulators of transcription by using their U3 regions.

In the present study, we analyzed the promoter activity of 3 loci of PERV LTR elements in human cell lines. The methylation status was studied in several tissues obtained from the National Institutes of Health (NIH) miniature pig. We have described the characterization of DNA methylation and sequences of 3 loci of PERV LTR elements.

MATERIALS AND METHODS

Genomic DNA isolation and PCR amplification

Genomic DNA was isolated from frozen tissues samples (liver, lung, thyroid, lymphatic gland, gall bladder, and stomach) of the NIH miniature pig by using a standard protocol. Polymerase chain reaction (PCR) amplification was performed using this DNA, and each PCR product was amplified using the primer set shown in Table 1 (Huh et al., 2007). Each amplification step was performed as follows: after an initial denaturation step at 94°C for 4 min, the genomic DNA was amplified for 30 cycles at 94°C for 40 s, 55°C for 30 s, and 72°C for 40 s.

Molecular cloning, sequencing, and data analysis

The PCR products were separated on a 1.5% agarose gel, purified with a QIAquick gel extraction kit (Qiagen), and cloned into the pGL4.11 vector (Promega). The cloned DNA was isolated using a plasmid DNA purification kit (Labopass). Sequencing was performed by the Macrogen.
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Table 1. PCR primers used for the amplification of long terminal repeat elements of porcine endogenous retrovirus

| Accession No. | Primers       | Sequences (5’-3’)                          | Products size (bp) |
|---------------|---------------|--------------------------------------------|--------------------|
| CT737311      | LTR-1-F       | 5’-AAG CCA ATC TCC CTT CTT CC-3’           | 748                |
|               | LTR-1-R       | 5’-TAT TTC CAT CCC TGA ACC CA-3’           |                     |
| CT797462      | LTR-2-F       | 5’-CTC AGT GGG TTG GGG TTC T-3’            | 904                |
|               | LTR-2-R       | 5’-TGC TTT CAC AAG TAT TCA TCA C-3’        |                     |
| CT827949      | LTR-3-F       | 5’-GGA GGG TAG GAC ACA GTG GA-3’           | 961                |
|               | LTR-3-R       | 5’-CTT GCA TTC GGC TTC TG-3’               |                     |

Table 2. PCR primers used for the high resolution methylation (HRM) analysis of long terminal repeat elements of porcine endogenous retrovirus

| Accession No. | Primers       | Sequences (5’-3’)                          | Products size (bp) |
|---------------|---------------|--------------------------------------------|--------------------|
| CT737311      | LTR-1-HRM-F   | 5’-GTA TAG GGT TTG TGA ATT TTA TAA AAG-3’  | 125                |
|               | LTR-1-HRM-R   | 5’-CAA AAA AAA AAT TTT TAT TCC AAC C-3’    |                     |
| CT797462      | LTR-2-HRM-F   | 5’-GGG TTT TGT GAA TTT TAT AAA AGT-3’      | 122                |
|               | LTR-2-HRM-R   | 5’-CAA AAA AAA AAT TTT TAT TCC AAC C-3’    |                     |
| CT827949      | LTR-3-HRM-F   | 5’-GTT GGT TTG GTG ATT TTA AAA GGT G-3’    | 118                |
|               | LTR-3-HRM-R   | 5’-ACA AAA AAA TTA TCT TCC AAC A C-3’      |                     |

Company (Korea) by using primer sets that corresponded to the pGL4.11 vector sequences pGL4.11F (5’-CTA GCA AAA TAG GGT GTC CCC AG-3’) and pGL4.11R (5’-CTT GGA GTG AGA ATG G-3’). Sequence alignment and comparative sequence analysis of the PERV LTR elements were performed using the BioEdit program. Transcription factor-binding sites in the PERV LTR element sequences were predicted using MATCH in TRANSFAC v8.0 (http://generegulation.com).

Cell culture and transient transfection assay

HepG2 and Hep3B cells (human hepatocellular carcinoma cells) were cultured at 37°C in a 5% (v/v) CO₂ incubator in Dulbecco’s modified Eagle’s medium that had been supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) antibiotic-antimycotic solution. The cells were plated in 24-well plates at a cell-density of 3 × 10⁴ cells/well and grown to 60% confluence. Then, the cells were transfected with mixtures that contained 100 ng of the pGL4.11 LTR plasmid (PERV LTR elements of LTR1, LTR2, and LTR3) and pGL4.11 basic vector that had been linked to luciferase (Promega) by using Lipofectamine Plus (Invitrogen) as described in the manufacturer’s protocol. In addition, 100 ng of pRL-TK plasmid vector was used to normalize the transfection efficiency. After 24 h of transfection, the cells were washed with Dulbecco’s phosphate-buffered saline and lysed in luciferase lysis buffer. The activities of firefly luciferase and Renilla luciferase in the cellular extracts were measured using a dual-luciferase reporter assay system (Promega) and a luminometer, respectively. Relative luciferase activity was obtained by normalizing the activity of the firefly luciferase with the Renilla activity. Each experiment was performed in triplicate.

HRM analysis

Two types of control DNA were prepared as methylated genomic DNA controls. The unmethylation control was amplified using an Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare). The full-methylation control was treatment of CpG methyltransferase (M.SssI, BioLabs). Each type of DNA (genomic DNA from 6 each tissues, methylated control DNA, and un-methylated control DNA) was quantified as being 500 ng by using a Nanodrop-1000 system (Thermo Scientific). Genomic DNA was bisulfite modified using an Imprint DNA Modification Kit (Sigma) as per the manufacturer’s instructions. Primer sets for each PERV LTR element that was specific for the bisulfite modified DNA were designed using the Meth primer program (www.urogene.org/methprimer), and their sequences are shown in Table 2. High resolution melting (HRM) analysis was performed on a Rotor-Gene Q (QIAGEN) to investigate the DNA methylation status. Each reaction consisted of 1 μl of bisulfite-modified DNA (30 ng) and the following constituents in the final concentrations and volumes mentioned: 5 μl of 2× EpiTect HRM PCR Master Mix (QIAGEN), 0.75 μl of 10 μM primer mix, and 3.25 μl of RNase-free water. The PCR cycling conditions for the PERV LTR elements were as follows: 95°C for 5 min, followed by 45 cycles of 95°C for 10 s, 59°C for 30 s, and 68°C for 10 s. Melting analysis was carried out over 65°C–85°C in 0.1°C increments every 2 s. Each sample was amplified in triplicate.
Calculation of methylation differences and statistical analyses

Differences in DNA methylation, as detected by performing HRM analysis, were calculated by employing net temperature shift (NTS) analysis. Subtraction of the methylated-control normalized curve from the normalized curve for each test was performed automatically within the Rotor-Gene Q program. The summed fluorescence value at each temperature point (0.1°C intervals) within the entire melt range was divided by 100 to obtain the average distance between the curves. A shift in a sample’s normalized melt curve to the left of the normalized melt curve of the methylated control indicated that the sample was less methylated. A plot showing the difference in the fluorescence value at each temperature point along each melt curve was obtained at 0.1°C intervals from 70°C to 80°C and totaled 100 readings.

RESULTS AND DISCUSSION

The LTR elements of the PERVs were examined using PCR amplification to detect any variations or specific features in the genome of the NIH miniature pig (Fig. 1). To determine whether the PERV LTR elements were active with respect to DNA methylation, we first examined the promoter activities of the PERV LTR elements of each locus by using a dual-luciferase reporter assay. The PERV LTR3 element showed the highest promoter activity, while the PERV LTR2 element exhibited the lowest promoter activity (Fig. 2). To determine whether these PERV LTR elements were active with respect to epigenetic regulation, we inspected their methylation status by using HRM analysis based on tissues of the NIH miniature pig. Compared to other PERV LTR elements, PERV LTR3 was less methylated than the PERV LTR1 and LTR2 elements in the liver tissue (Fig. 3). Here, the PERV LTR elements were more methylated than in the other tissues of the NIH miniature pig. Other tissues like those of the lungs, thyroid, lymph nodes, gall bladder, and stomach also did not exhibit a high degree of methylation of over 50% (Fig. 3). Thus, in porcine tissues, the PERV LTR elements are controlled by methylation events to protect their biological activities. By using multiple alignments and TRANSFAC v8.0, we compared PERV LTR elements structure and transcription factor-binding sites between the LTR elements. Their sequences showed highly conserved structures; however, very high frequencies of insertion and deletion (INDEL) events were especially observed in the U3 region. Tandem repeat sequences in the U3 region are known to be key regulators of transcription. Thus, these data suggest that the PERV LTR elements in the NIH miniature pig genome are also regulated by trans-acting sequences in the U3 region.

PERV LTR elements are known to have promoter activ-

![Fig. 1. PCR amplification of long terminal repeat elements of porcine endogenous retroviruses from the liver tissue of an NIH miniature pig. Solitary long terminal repeat (LTR) elements of porcine endogenous retroviruses (PERVs) on several loci (1, PERV-LTR1; 2, PERV-LTR2; and 3, PERV-LTR3). Each PERV LTR polymerase chain reaction (PCR) products indicate 748-bp, 904-bp, and 961-bp.](image)

![Fig. 2. Promoter activities of long terminal repeat elements of the porcine endogenous retrovirus in the hepatocellular carcinoma cell lines HepG2 and Hep3B. Three of the PERV LTR elements were transiently transfected into HepG2 cells and Hep3B cells and were then used in dual-luciferase assays. The relative luciferase activity is shown in the schematic diagram where the results are expressed as the ratio of the luciferase activity to that of the promoter-less pGL4.11 reporter plasmid. The relative luciferase activity was obtained by normalizing the activity of firefly luciferase with Renilla activity. Each experiment was performed in triplicate. The average and standard errors (error bars) have been presented.](image)
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and could regulate the transcription of proviruses. The PERV-A and PERV-B families infect human cells in vitro. Thus, they could lead to serious complications in xenotransplantation (Martin et al., 1998). Recently, research on the relationship between DNA methylation and promoter activity has been actively discussed. However, only a few reports are available on active PERV expression with respect to DNA methylation analysis (Park et al., 2010). In previous studies, several subtypes of PERV LTR elements showed different promoter activities; by changing DNA methylation through treatment with M.SssI, the promoter activity of PERV LTR was downregulated (Park et al., 2010). Thus, changes in the DNA methylation patterns can affect PERV LTR promoter activity. In this study, we examined the relationship between DNA methylation and the promoter activities of PERV LTR elements in a specific locus of the pig genome. We observed that the PERV LTR3 element, which was less methylated than other loci, had very strong promoter activity (Figs. 2 and 3). Generally, DNA methylation affects the regulation of gene expression. The difference in DNA methylation appeared to be specific for the cell type, tissue type, or developmental stage. Thus, DNA methylation plays a crucial role in the development of nearly all types of cancer (Jaenisch and Bird, 2003). DNA methylation occurs in CpG islands that spread in repeat elements. PERV LTR elements have repeat sequences and therefore have many CpG islands. These characterizations permit methyltransferases to methylate cytosine residues. Consequently, methyl cytosine affects the transcription of genes by impeding the binding of transcriptional proteins or the recruitment of methyl-CpG-binding domain proteins (Choy et al., 2010). PERV LTR elements have several transcription factor-binding sites and CpG islands (Park et al., 2010; Jung et al., 2013). These data suggest that differences in DNA methylation according to each locus and tissue could affect the promoter activity of each PERV LTR element.

However, interestingly, the methylation status and promoter activity differed according to the breed of pig, although the PERV LTR elements occupied the same locus in the porcine genome. The promoter activity and methylation status of the PERV LTR1, 2, and 3 elements were investigated in individual hybrids (KYP1-6) of KDP (Korean domestic pig) × Yorkshire (Huh et al., 2007). In these hybrids, the PERV LTR1 element showed strong promoter activity and strict hypermethylation in various tissues, but the PERV LTR3 element exhibited less promoter activity and hypermethylation status. Thus, depending upon the breed, each PERV LTR element showed different molecular characteristics for the same locus. The PERV sequences showed breed-dependent differences in the expression, copy number, and chromosomal distribution of the sequences (Jin et al., 2000; Wilson, 2008). However, these results still do not indicate which breed is better for xenotransplantation. Therefore, continued screening research is necessary.

In recent times, DNA methylation research has become an interesting topic in terms of gene expression regulation. However, studies on the DNA methylation of PERVs have not yet been conducted. Such epigenetic research is very important for understanding the characteristics of PERVs. Our data show that each PERV LTR element exhibited different promoter activity and different methylation status depending on the locus and type of tissue (Figs. 2 and 3). These results could not be used to determine whether DNA methylation regulated the promoter activities of PERV LTR elements, but we believe that the results indicate that promoter activity was affected. Other reports also underpinned that several chemical treatments that bring about changes in DNA methylation also change their promoter activity. In human cells and mouse cells, DNA methylation analysis

Fig. 3. Quantification of differences in methylation by using net temperature shift. DNA methylation was investigated using high resolution melting (HRM) analysis. The long terminal repeats (PERV-LTR) PERV-LTR1 (A), PERV-LTR2 (B), and PERV-LTR3 (C) of porcine endogenous retroviruses showed methylation. The normalized fluorescence at each temperature point on the melt curve was used to calculate the mean net temperature shift (NTS). The Y-axis of the bar graph indicates the degree of methylation. Tissue samples used for (A), (B), and (C) are as follows: 1, liver; 2, lung; 3, thyroid; 4, lymph node; 5, gall bladder; and 6, stomach. Each experiment was performed in triplicate. The average and standard errors (error bars) have been presented.
with LTR elements is a dynamic process. Analysis of the promoter activity regulation of human endogenous retrovirus LTR elements by using M.SssI treatments showed that the activity is effectively suppressed by regulating DNA methylation (Lavie et al., 2005). M.SssI treatment also showed that regulation of the activity of PERV LTR elements was influenced by DNA methylation (Park et al., 2010). On the basis of the information obtained from these results, it is possible to regulate the transcriptional activity of PERV LTR elements by controlling DNA methylation with appropriate chemicals.

The structural analysis indicated that each LTR element had various structures and sequences in the U3 region. Tandem repeat sequences, in particular, are known to affect the promoter or enhancer activities of LTR elements (Scheef et al., 2001). A 39-bp repeat sequence exists in the U3 region: it comprises a tandem repeat that contains 2 of the original 18-bp repeat and original 21-bp repeat sequences. However, each LTR element has a distinct number of repeat sequence copies. As shown in Fig. 4, the PERV LTR1 and LTR2 elements have 2 of the tandem repeat sequences with the original 18-bp repeat, but the PERV LTR3 element has only 1 copy of the tandem repeats with the original 18-bp repeat in the U3 region. However, the sequences that are downstream from the arrow showed a high degree of conservation (Fig. 4), and their sequences were included in the R and U5 regions. In previous studies, the U3 region was found to more strongly affect promoter activity than the well-conserved R and U5 regions (Della Chiara et al., 2011; Mukerjee et al., 2010). Thus, these variations in the U3 regions of PERV LTR elements could affect their promoter activity. In this study, the PERV LTR3 element showed strong promoter activity; however, it had only 1 copy of tandem repeats in the U3 region (Fig. 4). Apart from deleted region of PERV LTR3 element compared with other PERV LTR elements structure included NF-Y binding site. NF-Y is such transcription factor that binds CCAAT motifs in the promoter regions of a variety of genes (Marziali et al., 1999). NF-Y is a ubiquitous heteromeric transcription factor that is composed of 3 subunits, NF-YA, NF-YB, and NF-YC, all of which are necessary for DNA binding (Santos-Rosa et al., 2002). It is not a strong activator but rather a promoter organizer that cooperates with the activity of neighboring transcription factors (Ceribelli et al., 2008). On the other hand, transcription factors only exist in PERV LTR3 elements, such as Nkx2-2 and Elk-1, where they act as transcription activators and initiate gene expression (Costa et al., 2011; Bernal-Mizrachi et al., 2001). Thus, PERV LTR3 elements have 2 deletion regions and have transcription activator-binding sites in their U3 regions. The strong promoter activity of the PERV LTR3 element may be affected by active transcription factor-binding sites.

PERV LTR elements that have strong transcription activity are similarly distributed throughout the pig genome. These results showed that studying active PERVs and regulation analysis is necessary for xenotransplantation research. Active PERV LTR elements

![Fig. 4](Image)

*Fig. 4.* Multiple alignments used to analyze the structure and transcription factor-binding sites of the long terminal repeat elements of porcine endogenous retroviruses. Three loci of PERV LTR sequences were multiple alignments that included the U3, R, and U5 regions with consensus sequences of the PERV LTR element. Each rectangular box indicates transcription factor-binding sites. The transcription factor binding sites were predicted (threshold > 0.95) using TRANFAC software (v8.0). The round brackets indicate the orientation of the sequence, and the square brackets indicate tandem repeat sequences such as original 18-bp and 21-bp sequences. The gray boxes include 39-bp tandem repeat sequences. Conserved R and U5 regions are indicated with starred arrows.
consist of tandem repeat sequences. Thus, they have CpG islands and several *cis*-element regulator sequences. These characteristics are elicited that chance to epigenetic changing and *trans*-element regulations. The other way, it should be possible to use these characteristics to regulate their activities. Thus, the analysis of various aspects of active PERV regulation is necessary.

Recently, evidence has been obtained regarding the presence of at least 2 viral subpopulations in gammal retroviruses from Eurasian and African host species (Nascimento et al., 2011). These evolutionary occurrences of viral recombination may be relevant to xenotransplantation risk analysis since these recombinants could favor adaptation and confer the capacity to maintain ongoing infection in somatic cells and germ cells (Nascimento et al., 2011). Thus, like this study also should be taken into account in xenotransplantation. Furthermore, approaching of chemotherapy is also very important. Inhibitors of HIV reverse transcriptase could be used against the PERV reverse transcriptase (Powell et al., 2000; Qari et al., 2001). However, protease inhibitors of HIV do not exert inhibitory effects on PERV protease (Blusch et al., 2002). Thus, novel protease inhibitors might offer the most promising option for antiviral protection against the potential risks of human PERV infection. Further studies are required for the application of protease inhibitors and reverse transcriptase inhibitors in combination to treat PERV infections that develop in human xenograph recipients.

In summary, regulation of transcription factor expression and treatment approaches involving changes in methylation for controlling the transcriptional activity of PERV LTR elements are readily achievable. For xenotransplantation, the transmission of PERVs continues to be a risk, and it is difficult to eliminate PERVs from pig tissues. Thus, understanding PERV LTR element sequences, which function as promoter or enhancer regulators for transcriptional activity, is very important and constitutes an interesting research topic.

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