Molecular characterization of long terminal repeat of porcine endogenous retroviruses in Chinese pigs

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Summary. – Pigs offer an unlimited source of xenografts for humans. However, vertically transmitted Porcine endogenous retrovirus (PERV) poses an infectious risk in the course of pig-to-human transplantation. In this study, we characterized PERV long terminal repeat (LTR) sequences from three species of Chinese pigs Banna minipig inbred (BMI), Wu-Zhi-Shan pig (WZSP), and Neijiang pig (NJP-A), and compared them with those of known PERVs (PERV-A, PERV-B, PERV-C, PERV-NIH, and 293-PERV-43). Genomic DNA extracted from peripheral blood mononuclear cells (PBMCs) of the Chinese pigs was used for PCR-amplification, cloning, and sequencing of LTRs. The sequences of BMI and WZSP LTRs were found identical with those of PERV-A and PERV-B, while that of NJP-A LTR was found close to those of PERV-C and PERV-NIH. The gammaretroviral nature of PERV LTRs from Chinese pigs was proved. These LTRs contained also promoter elements including enhancer-like repeats comparable with those of other PERVs. These findings suggested that PERVs from Chinese pigs were similar to PERV-A and PRV-B. Moreover, this study provided new data for the evaluation and selection of pigs to be used in the xenotransplantation.

Keywords: xenotransplantation; Porcine endogenous retrovirus; long terminal repeat; sequence alignment; phylogenetic analysis

Introduction

The ongoing shortage of available human organs for transplantation with the resulting long waiting time and waiting-list patient deaths is spurring the examination of other means of treating organ failure (Sandrin and McKenzie, 1999). Clinical trials are in progress to test the feasibility of porcine cells or tissues as an alternative means (Levy *et al.*, 2000). However, the application of porcine cells to human would bring the possibility of introducing an infectious agent from the pig into the xenograft recipient and even a spread to the human population (Chapman, 1995). PERVs residing in the pig genome and showing similarities to type C of retroviruses of other species were described almost 30 years ago (Armstrong *et al.*, 1971). Endogenous retroviruses are present in the genomes of all vertebrate species. However, most copies are truncated or mutated and consequently, their replication is incompetent. Only a minority of proviruses are functional as reported for the pigs (LeTissier *et al.*, 1997). Infectious PERV particles including two subtypes PERV-A and PERV-B are produced by porcine cell lines, tumors, aorta endothelial cells, and PBMCs (Wilson *et al.*, 1998). In the worse case scenario, it is feared that PERV transmission to the humans might be the starting point of a man-made threat to the health of xenotransplant patients as well as general public (Boneva *et al.*, 2001; Scobie *et al.*, 2009). In theory,

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Abbreviations: BMI = Banna minipig inbred; LTR(s) = long terminal repeat(s); PBMC(s) = peripheral blood mononuclear cell(s); PERV = Porcine endogenous retrovirus

PERVs share the pathogenic potential of retroviruses that includes insertional mutagenesis and immunosuppression by themselves or after a recombination with human retroviruses (Niebert *et al.*, 2005). Many retrospective studies of patients who had been treated with porcine cells and tissues showed no evidence for the transmission of PERV (Paradis *et al.*, 1999; Gu, *et al.*, 2008; Valdes-Gonzalez, *et al.*, 2010). In contrast, a recent study revealed that after the transplantation of pig pancreatic islets to the NOD/SCID mice, a PERV infection was detected in several tissues, what indicated a xenozoonotic potential of those retroviruses (Van der Laan *et al.*, 2000). However, there has been no evidence of a PERV transmission in the nonhuman primate xenotransplantions performed to date.

A complete PERV genome includes *gag, pol, env* genes, and 5', 3'LTRs. The product encoded by *gag* is a core protein containing many highly conserved domains that are called a group specific antigen. The *pol* gene encodes various enzymes essentially important in the virus life cycle including 3 important enzymes, e.g. protease, integrase, and reverse transcriptase. *Env* gene encodes envelope proteins that determine a host tropism of the virus (Herring *et al.*, 2001). The existence of LTR in viral promoter that often acts as an enhancer element is well known (Yoshimura *et al.*, 1999; Huh *et al.*, 2009). In addition, there are two different types of LTRs that significantly affect the replication properties of single viruses by involving a special set of transcription factors (Tonjes *et al.*, 2003). Both PERV-A and PERV-B proviruses demonstrate LTRs that harbor repeats at the U3 region.

Since the studies about the LTRs from Chinese pigs have not been reported yet, the PERV LTR sequences from three species of Chinese pigs including BMI, WZSP, and NJP-A were characterized and compared with those of known PERVs (PERV-A, PERV-B, PERV-C, PERV-NIH, and 293-PERV-43). Thus, the knowledge of PERVs in Chinese pigs could be extended.

Materials and Methods

Genomic DNA isolation. The sequences analyzed here included 10 Chinese pigs, Banna minipig inbreed (BMI465, BMI0527, BMI0551, BMI0595, BMI650, BMI652, BMI0674, BMI4410), Wu-Zhi-Shan pig (WZSP-1), Nei Jiang pig (NJP-A), which were from different provinces of China. PBMCs were isolated from three Chinese pigs. Genomic DNA was extracted from the cells by blood genomic DNA kit (Pharmacia).

PCR, cloning, and sequencing. The primer was designed from the region of PERV LTR P9/P10 with upstream 5'-TGCAACCTAACCCTCCCAGA AC-3' and downstream 5'-GAACATAAGGGACTCCACCAAC-3'. The standard PCR program was one cycle of 94°C for 5 mins, 35 cycles of 94°C for 1min, 64°C for 1min, and 72°C for 2 mins and 1 cycle of 72°C for 10 mins. The approximately 1.2 kb PCR products were extracted with gel extract kit (Qiagen) and cloned into pGEM-T vector (Promega). All obtained clones were sequenced by the use of ABI prism. To analyze the relationship between PERV in Chinese pigs and other reported PERVs, nucleotide sequences of PERV-A, PERV-B, PERV-C, PERV-NIH, PERV-PK15, and 293-PERV-43 were obtained from GenBank. The nucleotide sequences of LTR from Chinese pigs have been submitted to the GenBank under Acc. Nos. DQ250029-DQ250038.

Sequence and phylogenetic analysis. The obtained LTRs sequences and other sequences from GenBank were analyzed by several soft wares. The Vector NTI was applied to locate the homology regions. The alignment of all sequences was done by the ClustalW software. A neighbor-joining phylogenetic tree was constructed from nucleotide sequences by the use of phylip 95/96 software.

Results

Structure of PERV LTRs

BLAST search of the GenBank DNA database revealed that the obtained sequences were of retroviral origin that showed more than 80% homology. The LTR sequences of representative members of each group were deposited in the GenBank.

The direct repeat in the PERV LTR consisted of 39 bp, which comprised an 18 bp subrepeat located upstream of a 21 bp subrepeat. In contrast, the subrepeats (18 bp and 21 bp) were separated from each other in the U3 region. LTR was present 5.5 times in the 293-PERV-43 and 2.5 times in PERV-A, PERV-B, PERV-PK15 (Fig. 1). In pig analysis, BMI4410 contained only one copy of 39 bp repeat and an extra 18 bp repeat, while the others pigs BMI465, BMI0527, BMI0551, BMI0595, BMI650, BMI 652, BMI0674, and WZSP-1 contained two copies of 39 bp repeat and an extra 18 bp repeat (Fig. 1). A similar sequences were found in PERV-C, PERV-NIH, and NJP-A representing 1.5 times of LTR.

Phylogenetic analysis of PERV LTRs

The classification of the LTR clones amplified in this study was carried out by the sequence comparison of clones to the several published PERV LTR sequences. Phylogenetic homology analysis was based on the nucleotide sequences (Fig. 2). In the resulting LTRs phylogenetic tree, we found two major clusters of PERV LTRs. A classification into PERV-A and PERV-B subfamilies were done according to the phylogenetic relationship of phylogenetic tree. The first cluster included most of the clones amplified in this study BMI0595, 0551, 0527, 0674, 650, 652,465, WZSP-1, as well as PERV-A, -B, -PK15, and 293-PERV-43. The compared PERVs from the GenBank belonged to the

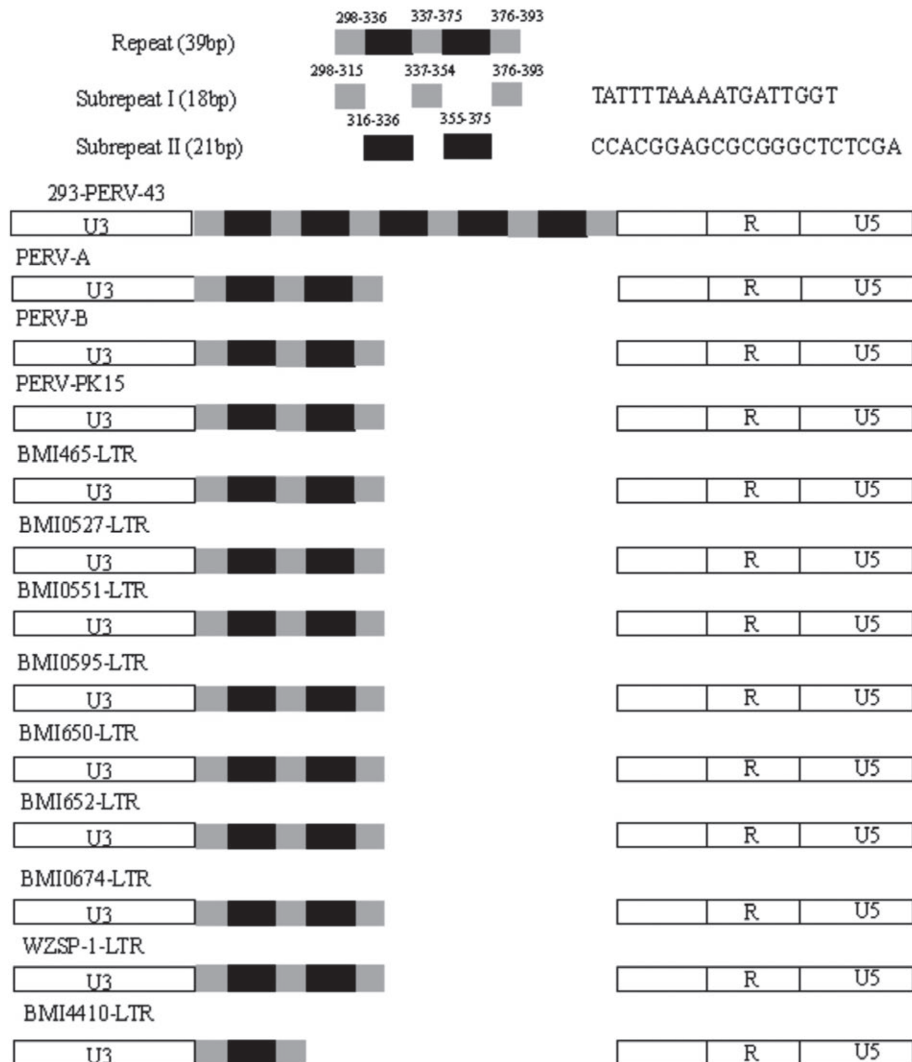

Fig. 1

Structure of PERV LTRs analyzed in this study

U3, R, and U5 regions (open boxes), 18-bp repeats (gray boxes), and 21-bp repeats (black boxes).

gammaretroviruses. As a result, we concluded that the sequences of several LTR clones from Chinese pigs were homologous to the gammaretroviruses. When multiple members of a single group were identified, the sequences from Chinese pigs were most closely related to the infectious 293-PERV-43 and PERV-PK15. Conversely, NJP-A was located in another cluster that also included PERV-C and PERV-NIH (Fig. 2).

Sequence analysis of PERV LTRs

The nucleotide sequences of the LTRs of known types of gammaretroviral PERVs obtained from the GenBank and LTR sequences of the analyzed pig viruses were compared in order to find some common feature. The strain 293-PERV-43 was obtained from the PERV-infected 293 cells, while the PERV-C was a recombinant genomic clone (Akiyoshi *et al.*, 1998). Infectious PERV-NIH was isolated from NIH miniature pig using 293 cells (Wilson *et al.*, 2000). the comparison showed that LTRs from the Chinese pigs were identical to those of other PERVs containing U3/U5/R region. Among the examined PERV LTRs, the highest homologies were found in the region R and U5. Ten PERV clones isolated from Chinese pigs could be divided to the two groups. The first group contained nine clones BMI465, 0527, 0551, 0595, 650, 652, 0674, 4410, WZSP-1, and the second one contained only one clone PERV NJP-A (Fig. 3).

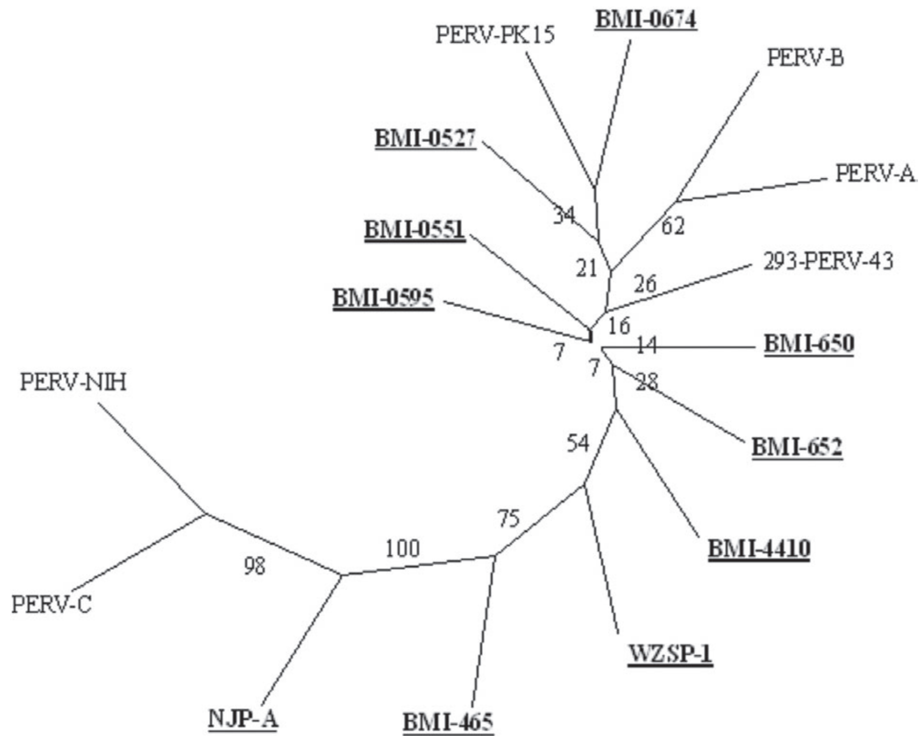

Fig. 2

Phylogenetic tree of PERV LTRs

The tree is based on the data from PERV LTR analysis of the different pig species including BMI0595, 0551,0527, 0674, 650, 652, 4410, 465,WZSP-1, and NJP-A. Numbers denote the percentage of 600 bootstraps in which a certain branch occurred.

The first group was identical to the PERV-A, PERV-PK15, and 293-PERV-43. The PERV-NJP-A clone showed lower degree of homology than the other BMI clones, when compared with PERV-A and PERV-B, what demonstrated a different structure within the U3 region (Fig. 3). The sequence of the 293-PERV-43 was identical to the nine Chinese clones except for three extra 39 bp repeat. The PERV-NJP-A showed more similarity to the PERV-C and PERV-NIH. In the previous study, PERV-A shared only 64% nucleotide identity with the PERV-C and PERV-NIH (Zhang *et al.*, 2004). Our study showed that the sequence differences were located primarily in the U3 region. A perfect 9 bp inverted repeat in the U5 region was present in PERV-A, PERV-PK15, BMI0527 LTR, BMI465 LTR, BMI4410 LTR, WZSP-1 LTR, and NJP-A LTR. The inverted repeat sequence of PERV-C and PERV-NIH has two nucleotide substitutions only. A 5'-ATAAA-3' box in the U3 region was identified in all analyzed LTR sequences and was demonstrated as a potential TATA box located 32 nt upstream of the transcription start site (Krach *et al.*, 2001). Similar to other gammaretroviruses and other reported PERVs, the LTRs from Chinese pigs also presented direct repeats in the U3 region, which resembled the enhancer element and AATAAAA polyadenylation signal in the R region.

Discussion

PERVs remain a major safety concern for porcine xenotransplantation despite of the fact that most copies present in the pig genome are likely to be replication defective (Ericsson *et al.*, 2001). The lower repeat number of LTRs is an adaptation to the acquired endogenous replication cycle of PERV. this LTR structure largely reduces the transcriptional activity, what is in line with the observations that artificially elongated LTRs kill their host cells, favoring lower repeat copy numbers in U3 (Krach *et al.*, 2001). However, in our research, we found that BMI4410 had less repeats, but corresponding PBMCs were capable of infecting 293 cells. On the other hand, the BMI0527 had 2.5 times more of LTR repeats and could not release the infectious PERV (Zhang *et al.*, 2006). Our previous studies have shown a high number of PERV copies in Chinese pigs and therefore, the extensive research for the additional PERV copies was carried out by the identification and phylogenetic analysis of other sequences from the GenBank. By nucleotide sequence analysis of the LTRs, we have identified the sequence characterization of 10 Chinese pigs of different breeds. All PERV LTRs had a high degree of identify with the sequences of other

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293-PERV-43-AJ298073  TGCAACCTAACCCTCCCAGAACCCAGGAAGTTAATAAAAAGCTCTAAATGCCCCCGAATTCCAGACCCTGCTGGCTGCCAGTAAATAGGTAGAAGGTCAC  [100]
BMI0527-LTR           ....................................................................................................  [100]
BMI465-LTR            ....................................................................................................  [100]
BMI4410-LTR           ..........................................G......................C..................................  [100]
WZSP-1-LTR            ....................................................................................................  [100]
PERV-A-AF546883       ....................................................................................................  [100]
PERV-PK15-AF038601    ....................................................................................................  [100]
NJP-A-LTR             ......................................C..........T...........T..T.CC..--------.......A...A...T  [92]
PERV-C-AF546885       ---------------T.C....T.---..C....GGG.....TA....TGTT.T..........GTT..T.CCT.--------.......A...A.T.G  [73]
PERV-NIH-AF546887     ........G..T.........................G...............T.................T.CC..--------.......A...A...T  [92]
      U3
293-PERV-43-AJ298073  ACTTCCTATTGTTCCAGGGCCTGCTATCCTGGCCTAAGTAAGAT--------AACAGGAAATGAGTTGACTAAT-CGCTTATCTGGATTCTGTAAAACTG  [191]
BMI0527-LTR           ............................................--------......................-..........................  [191]
BMI465-LTR            ............................................--------......................-..........................  [191]
BMI4410-LTR           ............................................--------......................-..........................  [191]
WZSP-1-LTR            ............................................--------......................-..........................  [191]
PERV-A-AF546883       ............................................--------......................-..........................  [191]
PERV-PK15-AF038601    ............................................--------......................-..........................  [191]
NJP-A-LTR             .....T.GC....TT.AACTA...------------------------AGA.GGGAA...CAG.CCCCTG.G.A..TGCC...A.GC---A.G.....T  [164]
PERV-C-AF546885       GT..TT.GC....TT.AAATA...--------------------------GG.AGTA....AG.CCC-TG.G.A.ATG.C...A.GC---A.G.....T  [142]
PERV-NIH-AF546887     ....TT.GC....TT.....T....T..TGCT.TGT.CA..AC.TTGTGGAAGGGGAA...CAG.CCCCTG.G.AT.TGCC...ATGC---T.G.....T  [189]

293-PERV-43-AJ298073  ACTGGCACCATAGAAGAATTGATTACACATTGACAGCCCTAGTGACCTATCTCAACTGCAATCTGTCACTCTGCCCAGGAGCCCACGCAGATGCGGACCT  [291]
BMI0527-LTR           ........................................................................T...........................  [291]
BMI465-LTR            .........................................................C..........................................  [291]
BMI4410-LTR           .................................A..............................................................A....  [291]
WZSP-1-LTR            ...................................................................................................C  [291]
PERV-A-AF546883       ....................................................................................................  [291]
PERV-PK15-AF038601    ....................................................................................................  [291]
NJP-A-LTR             CT..AA..T.CTTG...TAAC.GGGA.AGGGTT.CTAA..GC.TGTT.GG..TCTG.AA.GCTGC.TG.AAAAGAT.AA..GAGGA.A..T.AATCC.TA  [264]
PERV-C-AF546885       CT..AA..T..T-------------------------------------..AG---------ATAA.A.GAA.AG-GGA.TTTC.AACTG.T.  [188]
PERV-NIH-AF546887     CT..AA..TGCTC---------------------CTAA..GC.TGTT.GG..TCTG.AA.CCTGC.TG.ATAAGAT.AA.AGAGGA.A..TCAATTG...  [268]

293-PERV-43-AJ298073  CCGGAGC TATTTTAAAA-TGATTGGTCCACGGAGCGCGGGCTCTCGA TATTTTAAAATGATTGGTCCACGGAGCGCGGGCTCTCGA TATTTTAAAATGAT  [390]
BMI0527-LTR           ....... ..........-............................. ....................................... ..............  [390]
BMI465-LTR            ....... ..........-............................. .......................A............... ..............  [390]
BMI4410-LTR           ....... ..........-............................. .................--------------------- -----.--------  [355]
WZSP-1-LTR            ....... ..........-............................. .......................A............... ..............  [390]
PERV-A-AF546883       ....... ..........-............................. ....................................... ..............  [390]
PERV-PK15-AF038601    ....... ..........-............................. ..................T.................... ..............  [390]
NJP-A-LTR             AACCGA. CCCAG...G.TC.G...CG...TAA.AT------GT.G.. A...C.G.T.AATA.ATCTTGGC..CAA.AT.TCTC.CC .GCCCAGG..CAGG  [358]
PERV-C-AF546885       GTTT... .TC.G.....C..G...CC...TAA..A------.G.T.. A..G..G.T.CACA.ATCTTGGT..CAA.AT.TCTC.CC C.CCCCG...CATG  [282]
PERV-NIH-AF546887     AAC.GA. CCCAG...G.TC.GG..TA....AA.-------------- -..G..G...CACA.ATCTTGGT..CAA.AT.TCTC.CC -.CCCCG...CATG  [352]

293-PERV-43-AJ298073  TGGTCCACGGAGCGCGGGCTCTCGA TATTTTAAAATGATTGGTCCACGGAGCGCGGGCTCTCGA TATTTTAAAATGATTGGTCCACGGAGCGCGGGCTCT  [490]
BMI0527-LTR           ....--------------------- --------------------------------------- ------------------------------------  [394]
BMI465-LTR            ....--------------------- --------------------------------------- ------------------------------------  [394]
BMI4410-LTR           ------------------------- --------------------------------------- ------------------------------------  [355]
WZSP-1-LTR            ....--------------------- --------------------------------------- ------------------------------------  [394]
PERV-A-AF546883       .......T................. ...................-------------------- ------------------------------------  [433]
PERV-PK15-AF038601    ....--------------------- --------------------------------------- ------------------------------------  [394]
NJP-A-LTR             CA----CAAACAT.TAACTC.AAA. C.A......T.A...........A...A........... .G...--------------------------------  [422]
PERV-C-AF546885       C.----CAAAT.T.TAACTCTAAA. C.A......T.A...........A............... AG...-----------------AAATTGA.T..T.TG  [362]
PERV-NIH-AF546887     C.----CAAAT.T.TAACTCTAAA. C.A......T.A...........A............... AG....----------------AAATTGA.T..T.TG  [433]
293-PERV-43-AJ298073  CGA TATTTTAAAATGATTGGT TTGTGACGCACAGGCTTTGTTGTGAACCCC[ATAAAA]GCTGTCCCGATTCCGCACTCGGGGCCGCAGTCCTCTACCCC--  [588]
BMI0527-LTR           --- ------------------ ........................................................................................--  [471]
BMI465-LTR            --- ------------------ ........................................................................................--  [471]
BMI4410-LTR           --- ------------------ -.......................................................................................--  [432]
WZSP-1-LTR            --- ------------------ ........................................................................................--  [471]
PERV-A-AF546883       --- ------------------ ........................................................................................GG  [512]
PERV-PK15-AF038601    --- ------------------ ........................................................................................--  [471]
NJP-A-LTR             --- -----.....C....... ........A..G.G.............................C.............T..........G...--  [513]
PERV-C-AF546885       T.. .C....G........... .....A.A..G.G......C.......................C...A.......................--  [531]
PERV-NIH-AF546887     T.. ......G........... .....A.A..G.G..............................C...A.......................--  [531]
            R
293-PERV-43-AJ298073  ------------ -------TGCGTGGTGTACGACTGTGGGCCCCAGCGCGCTTGG[AATAAAAA]TCCTCTTGCTGTTTGCATCAA GACCGCTTCTCGTGAG  [669]
BMI0527-LTR           ------------ -------......................................................................... ................  [552]
BMI465-LTR            ------------ -------......................................................................... ................  [552]
BMI4410-LTR           ------------ -------.......C..T............................................................. ................  [513]
WZSP-1-LTR            ------------ -------......................................................................... ................  [552]
PERV-A-AF546883       GGCCGCAGTCCT CTACCCC......................................................................... ...........T....  [591]
PERV-PK15-AF038601    ------------ -------......................................................................... ----------------  [536]
NJP-A-LTR             ------------ -------.......C...G..G......................................................... ................  [594]
PERV-C-AF546885       ------------ -------......................................................................... ................  [541]
PERV-NIH-AF546887     ------------ -------......................................................................... ................  [612]
                                                                                                                 U5
293-PERV-43-AJ298073  TGATTT-GGGGTGTCGCCTCTTCCGAGCCCGGACGAGGGGATTGTTCTTTTACTGGCCTTTCA--  [732]
BMI0527-LTR           ......-........A................................................TT  [617]
BMI465-LTR            ......-.........................................................TT  [617]
BMI4410-LTR           ......-.........................................................TT  [578]
WZSP-1-LTR            ......-.........................................................TT  [617]
PERV-A-AF546883       ......-.........................................................TT  [656]
PERV-PK15-AF038601    ------------------------------------------------------------------  [536]
NJP-A-LTR             ......-....................T...............T.C...C.G............TT  [659]
PERV-C-AF546885       .....AA....A.......T..........T...G.---------T.CT....G....T...A..TT  [598]
PERV-NIH-AF546887     .....AA....A.......T..........T...G.---------T.CT....G..A.T...A..TT  [669]
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Fig. 3

Sequence alignment of PERV LTRs

293-PERV-43 LTR (reference sequence), identical nucleotides (dots), different nucleotides (letters), missing nucleotides (dashes), and non-consensus TATA sequences (boxes).

PERVs obtained from the GenBank. Nine pigs of the first group were similar with PERV-A and PERV-B, while PERV LTR sequences of NJP-A pig were identical with those of PERV-C and PERV-NIH. The NJP-A, PERV-C differed from those of PERV-A, BMI and from each other mainly in the U3 region upstream of the repeats and in the U5 regions. In our research, NJP-A had 80% sequence identity with that of the PERV-C and PERV-NIH, with the majority of the nucleotide differences located in the U3 upstream and the U5 regions. The transcriptional activities of the PERV-A, PERV-B, and PERV-C LTRs relative to each other were similar in different cell types of different animal species as determined by transient expression assays (Wilson *et al.*, 2003). In our study, several Chinese pigs had similar sequence in LTRs compared with PERV-A. So, we predict the PERVs from these Chinese pigs might have some similar transcriptional and infecting characteristics with PERV-A and PERV-B, but an extensive research is needed to prove this assumption. In previous research, the distance of PERV repeats had a region of substantial size and contained sequences important for both the transcriptional regulation and pathogenicity of some murine leukemia viruses (Reuss *et al.*, 2001). The 39 bp repeat (18 and 21 bp subrepeats) box in U3 was identified, which multimerized dynamically upon replication and acted as a viral enhancer (Niebert *et al.*, 2005). The preliminary study of the 39 bp repeat indicated that it contained motifs for nuclear binding factors, similar to the repeat structures in LTR of murine leukemia virus (Lamins, *et al.*, 1984). The existence of repeats in type C retroviruses are well-known and they are described for the murine leukemia virus, human T-lymphotropic viruses 1 and 2, as well as murine endogenous retrovirus (VanBeveren, *et al.*, 1982; Paskalis *et al.*, 1986; Wolgamot *et al.*, 1999). In analyzed Chinese pigs, eight pigs from the first group and one pig from the second group bearing two and one 39 bp repeats, respectively, yielded proviral LTRs that showed reduced number of 39 bp repeats compared with the previous study on 293-PERV-43 (Zhang *et al.*, 2006). It was reported that 293-PERV-43 containing up to five copies of 39 bp repeat showed a strong promoter activity in the human and mammalian cell lines (Scheef *et al.*, 2001). They also demonstrated that the dislocated repeat box increased the activity of deletion mutant, irrespectively of location and orientation, which is an additional proof of the enhancer properties of repeat box (Scheef *et al.*, 2001). Additional copies of repeats detected in the LTRs would lead to the increase of virus replication in target cells and to the enhancement of virus-induced pathogenicity. In murine gammaretroviruses, the enhancer elements are present as direct repeats of 50 to 120 nts (Paskalis *et al.*, 1986). More reports have demonstrated that the multiple repeats are important determinants of not only for the viral replication, but also for disease specificity and latency of disease induction. The functional role of such repeats as an enhancer is a common feature (Fan, 1990). In Rous sarcoma virus LTR, the deletion of enhancer elements led to a 94% reduction of the original promoter activity (Cullen, *et al.*, 1985). In other report, there appeared to be a direct correlation between the number of repeats in U3 and transcriptional activity (Wilson *et al.*, 2003). The variation of repeat boxes in Chinese pigs also had an impact on the transcriptional activity of LTRs and accordingly on the level of replication capacity in these pigs. However, the nature of any infection or disease caused by interspecies retroviral infection cannot be predicted.

The phylogenetic analysis of PERV LTRs indicated that the elements could be classified into two subfamilies, which could be differentiated by the sequences amplified by the LTR specific primers. The BMI0595, 0551, 0527, 0674, 650, 652, 465, 4410, WZSP-1, and PERV-A, -B, -PK15 and 293-PERV-43 were clustered, whereas the other clones including NJP-A and PERV-C, PERV-NIH were located on another branch. The NJP-A showed an overall lower level of homology than other clones that was reflected by the phylogenetic distances.

Our nucleotide sequence alignment analysis of the LTRs of three different species of Chinese pigs revealed a structure characterization of basal promoter elements that was shared with other PERVs including PERV-A, -B, -C, -PK15, and –NIH. The repeats and transcription factor-binding sites were conserved in PERV LTRs. This type of information is helpful in the identification of PERV LTR regions essential for PERV replication in cells of different types. Our data also identified the ATAA non-consensus TATA box in analyzed Chinese pigs and also other PERV sequences that were demonstrated as the transcriptional activation in 293 cells. The deletion of these elements reduced the transcriptional activity by 50-fold.

In this report, we described the cloning and the molecular characterization of ten LTRs sequences derived from the genomes of three species of Chinese pigs. The identification of replication-competent retroviral copies in the pig genome would be helpful in identification of a pig breed, which produce a lower level of PERV load. In general, the number of active PERV copies is probably dependent on the particular animal species. If the functional copies of PERV are limited in the pig genome, the cloning of PERV-free pigs would become possible. We hope that the sequence data presented here will be a valuable tool for the comparison of PERV distribution in the different pig breeds and in the effort to rule out potentially infectious proviruses.

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