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Concise classification of the genomic porcine endogenous retroviral γ1 load to defined lineages

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Abstract

We investigated the infection history of porcine endogenous retroviruses (PERV) γ1 by analyzing published *env* and LTR sequences. PERV sequences from various breeds, porcine cell lines and infected human primary cells were included in the study. We identified a considerable number of retroviral lineages indicating multiple independent colonization events of the porcine genome. A recent boost of the proviral load in an isolated pig herd and exclusive occurrence of distinct lineages in single studies indicated the ongoing colonization of the porcine genome with endogenous retroviruses. Retroviral recombination between co-packaged genomes was a general factor for PERV γ1 diversity which indicated the simultaneous expression of different proviral loci over a period of time. In total, our detailed description of endogenous retroviral lineages is the prerequisite for breeding approaches to minimize the infectious potential of porcine tissues for the subsequent use in xenotransplantation.
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Keywords: ERV; PERV; Evolution; Xenotransplantation

Introduction

Endogenous retroviruses (ERV) are vertically transmitted proviruses derived from the retroviral infection of host germ line cells. The diploid single-stranded RNA genomes are reversely transcribed and integrated into the host genome as proviruses. They contain less than 10 kb and consist of the genes *gag*, *pro/pol* and *env* flanked by two long terminal repeats (LTR) which are identical at the time point of integration into the host genome (Gifford and Tristem, 2003; Coffin et al., 1997). ERV have been found in all vertebrates examined and cover 8% of the human and mouse genome (Lander et al., 2001; Waterston et al., 2002). The amplification of retroviral copies in the host genome primarily occurred by re-infection which gave rise to provirus families (Belshaw et al., 2004). Additional mechanisms of ERV amplification in the host genome have been discussed for distinct ERV families (Belshaw et al., 2005; Costas, 2002).

Mutations of retroviral genomes are caused by diverse mechanisms. During DNA synthesis, the low fidelity of retroviral reverse transcriptase generates point mutations and swapping between the diploid RNA genome results in hybrid proviruses (Galetto and Negroni, 2005). After integration into the host genome, point mutations and recombinations occur during mitosis and meiosis (Hughes and Coffin, 2005). Recombinations between corresponding 5′- and 3′-LTR produce single LTR which represent most of the proviral loci in some ERV families (Lopez-Sanchez et al., 2005).

Depending on their impact to the host, ERV underlie negative, neutral or positive selective pressure. Negative selection results in the extinction of ERV or host during evolution, whereas neutral selection causes loss of ERV function during host evolution. Functional ERV genes and/or proviruses which have been found in several species including human, koala, mouse, pig and sheep might be maintained by positive selection due to synergistic effects of ERV to the host over a longer period of time (de Parseval and Heidmann, 2005; Magre et al., 2003; Palmarini et al., 2004; Stoye, 1998; Tarlinton et al., 2006).

ERV sequences have been used for the investigation of the host genome evolution whereas few analyses have been done on the evolutionary history of ERV genomes itself (Costas, 2001; Hughes and Coffin, 2004; Lopez-Sanchez et al., 2005). For the porcine (*Sus scrofa*) endogenous retrovirus (PERV) family γ1 numerous sequences of distinct origin (pig breeds, infected

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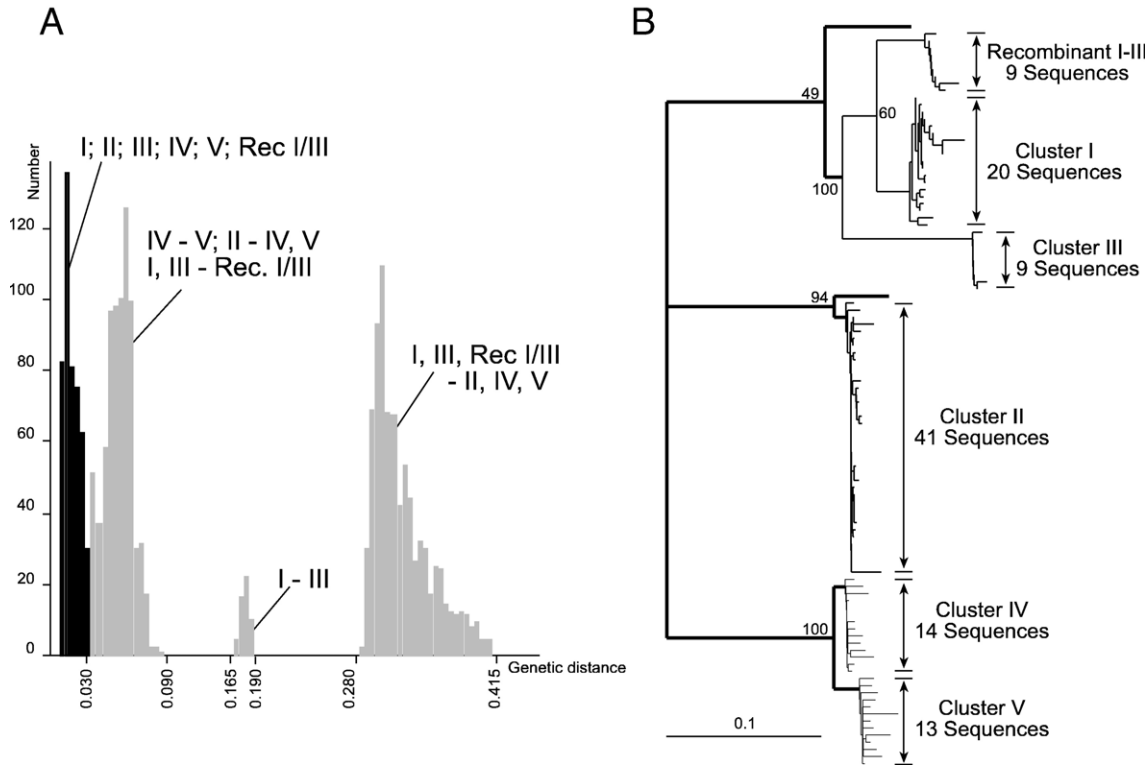

Fig. 1. Cluster analysis of PERV γ1 LTR sequences. 108 Non-redundant LTR sequences were analyzed by genetic distance distribution (A) and phylogenetic trees (B) and revealed five independent (I to V) and one recombinant (I–III) cluster. LTR of clusters I, II and III corresponded to PERV γ1A, B and C, respectively. (A) The genetic distance indicated on the abscissa was partitioned into units of 0.005 width and plotted versus the number of distance values in the respective segments. The black peak defined clusters, and the grey peaks showed the distance between the respective clusters. The relationship within and between the clusters is indicated. (B) The phylogenetic tree was based on a single neighbor joining tree and validated by bootstrapping and maximum parsimony (see Methods). The clusters defined by the genetic distance distribution analysis are indicated by bold lines and bootstrap values.

porcine and human cells, RNA transcripts) are available due to its potential infectious impact in xenotransplantation (Magre et al., 2003). PERV γ1 consists of the subfamilies A, B and C which have been defined by their different host tropism. The pig genome contains about 50 PERV γ1 copies with differences in the PERV load between breeds and individuals similarly to other species (Jin et al., 2000; Tomonaga and Coffin, 1998; Niebert and Tonjes, 2003a). PERV γ1C sequences were assumed to be derived from exogenous retroviruses (Martin et al., 2006; Wood et al., 2004). Recently, chromosomally assigned PERV γ1C proviruses were described (Hector et al., 2007). In addition to intact proviruses and mutant sequences, recombination events were found in PERV γ1 sequences (Klymiuk et al., 2003; Lee et al., 2002; Oldmixon et al., 2002) giving rise to potential patchwork repair of defect ERV sequences within and/or between species.

Here, we examined the infection history of the published PERV γ1 *env* and LTR sequences. We found evidence for the long-term proliferation and ongoing fixation of PERV γ1 lineages in the pig genome. The genomic PERV γ1 load was concisely classified into defined endogenous retroviral lineages which emerged from numerous master elements during re-infection events.

Results

Terminology

PERV have been classified to the subfamilies A, B and C by infectivity assays. Subsequently PERV genomes have been assigned to the respective infectious potential defined by their *env* gene. However, the classification of recombined or truncated PERV genomes to the defined subfamilies is complicated. In contrast to the biased nomenclature of retroviral sequences deduced from their infectious potential, we chose an unbiased classification solely defined by the phylogeny of the sequences. Our terminology enabled the concise definition of sequence clusters throughout the PERV genome while the classification to the subfamilies A, B and C was extrapolated from the *env* gene. To facilitate the correlation of our phylogenetic terminology to the

Fig. 2. Phylogeny of independent PERV γ1 LTR clusters I to V. Phylogenetic trees were created as described (see Methods). Representative LTR from the other clusters were used as outgroup for the generation of the trees. 5′- and 3′-LTR sequences from proviruses are indicated with the GenBank accession number or the BAC clone number followed by the GenBank accession number in parenthesis. LTR sequences from RNA transcripts were merged to artificial LTR and depicted in italics. The informative positions (as solid bars) in the 702-nt alignment, the number of singletons and the number of 18- and 21-mers in the U3R region (Wilson et al., 2003) are given for each LTR. Polymorphic nucleotides shared by the LTR from different proviruses but not by corresponding 5′- and 3′-LTR are shown with dotted bars. The position of the U3R region in the nucleotide alignment is indicated. *The sequences AY099323 and AY099324 were generated each from two independent PCR amplificates.

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