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Review Article

Detection of porcine endogenous retrovirus in xenotransplantation

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ABSTRACT

Xenotransplantation can provide a virtually limitless supply of cells, tissues and organs for a variety of therapeutic procedures. Cells and tissues for use in human transplantation procedures could be supplied using material taken from pigs. However, there is a potential risk of transmission of porcine infectious agents, including porcine endogenous retroviruses (PERVs), to a novel human host, with as yet unknown consequences. Three subtypes of PERV have been identified, of which both PERV-A and PERV-B have the ability to infect human cells *in vitro*. The third subtype, PERV-C, does not show this ability. Recombinant PERV-A/C forms have demonstrated infectivity in human cell culture. Monitoring in xenotransplantation should comprise screening of the source pig herd (PERV-A and PERV-B level expression assessment, PERV-C detection) and screening of recipients (differentiation between PERV transmission and chimerism). The detection of PERVs includes analyses of both DNA and RNA (PCR and RT-PCR), quantitative determination of the level of PERV nucleic acids (real-time PCR and real-time RT-PCR), assessment of reverse transcriptase (RT) activity (RT assays) and viral and recipient protein detection (immunological methods).

In summary, all available methods should be used in monitoring of PERVs in xenotransplantation, and caution should be exercised at all stages of monitoring. Such monitoring has enormous significance for eliminating the possibility of transmission of PERV infection, thus contributing to higher levels of safety in xenotransplantation.

1. PERV: potential risk in xenotransplantation

The limited availability of human organs and tissues for transplantation has prompted the search for alternative sources [1–3]. Xenotransplantation offers the promise of providing cells, tissues and organs for human recipients. According to the United States Public Health Service, xenotransplantation includes any procedure that involves the transplantation, implantation or infusion into a human recipient of either (a) live cells, tissues or organs from a nonhuman animal source or (b) human body fluids, cells,

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tissues or organs that have had *ex vivo* contact with live nonhuman animal cells, tissues or organs [4]. The comparable sizes of human and porcine organs, similar physiological parameters, ease of breeding pigs and production of transgenic pigs with a knocked-out galactose-alpha-1,3-galactosyltransferase gene locus (GalT-KO pigs) preventing graft rejection mean that the pig is a potential donor of material for xenotransplantation [5–7]. Moreover, the significant phylogenetic distance between pigs and humans reduces the risk of transmission of viral infections, and screening and qualified pathogen-free breeding diminish the risk of other zoonotic infections [8]. It is not known whether the therapeutic use of porcine material is completely safe because of porcine endogenous retroviruses (PERVs) present in all pigs [9–11]. The genetic material of PERVs, RNA, is transcribed by reverse transcriptase (RT) into DNA, and this can be integrated into the host DNA as a provirus [12]. Thus, PERVs are permanently embedded in the genome of all pigs [13]. There are three subtypes of PERVs: PERV-A, PERV-B and PERV-C. PERV-A and PERV-B are present in all pigs, and they are polytropic viruses able to infect human cells. The third subtype, PERV-C is an ecotropic virus, which infects only porcine cells [14]. PERV-A can recombine with PERV-C, thereby increasing its infectivity in humans [15]. PERV-C is not present in all pigs. Thus, screening herds for its presence is necessary to exclude the risk of PERVA/C transmission.

In view of the PERV infection risk, the International Xenotransplantation Association recently recommended careful screening of source pig herds for PERVs (selection of pigs that do not contain PERV-C in their germ line) to prevent recombination with PERV-A and selection of pigs that exhibit low-level expression of PERV-A and PERV-B, in addition to screening of recipients for PERV transmission using assays that are sufficiently sensitive to enable differentiation between transmission and chimerism [7,9].

2. Screening of the source pig herd for PERVs

Careful screening of the source pig herd destined for xenotransplantation should include PERV-C detection. This ecotropic subtype of virus is not present in all pigs. In Chinese experimental miniature pigs, PERV-A and PERV-B subtypes were present in the genome of all individuals, whereas the PERV-C subtype was detected in only 30% of pig genomic DNA samples [13]. Similarly, in conventional crossbred pigs, 23.5% (48/204) of pigs were positive for PERV-C, but the percentage of positive pigs varied between breeds from 9.1% to 54.3% [16]. In transgenic Polish Landrace pigs, 47% were PERV-C positive [17]. All three subtypes of PERVs are able to infect cells in a receptor-independent manner [18]. As mentioned above, PERV-C is not able to infect human cells. However, mutations in the envelope protein may produce a human-tropic variant of PERV-C [19]. Moreover, PERV-C can recombine with PERV-A, yielding recombinants (PERV-A/C) of unknown variants. Many studies have shown the ability of these recombinant viruses to infect human cells [11,20,21]. PERV-A/C found in normal pigs were replication competent [22]. They were integrated in the genome of somatic cells but not in the germ line. PERV-A/C recombinants contain a reverse transcriptase region derived from PERV-C, resulting in increased RT activity compared to that of PERV-A [23]. This can lead to significantly higher infectivity of PERV-A/C. *In vitro* studies on human cell lines have shown that the viral titre of PERV-A/C increases due to genetic alternations in the LTR (long terminal repeats), similar to those found in the PERV-A LTR [24]. In addition, PERV-A/C positive pigs showed increased occurrence of clinical disease compared to healthy individuals [16]. There are no data on pig-to-pig transmission of PERV-C. Inoculation of pigs with high-titre PERV-A/C did not cause provirus integration [25]. The study suggested that the prevention of infection with PERV-A/C may be due to receptor interference, elimination of innate immunity or intracellular restriction factors preventing virus replication [25]. Although PERV-A/C recombinants do not infect inoculated pigs, possible pig-to-pig transmission of PERV-C and therefore recombination with PERV-A cannot be excluded. Thus, screening pig herds for the presence of PERV-C is essential for preventing humans from potentially hazardous consequences, both for individual patients and public health.

To screen pigs for the presence of PERV-C, different methods have been developed, including standard PCR methods, sensitive nested PCR and real-time PCR [26]. For RNA detection, the PCR method preceded with reverse transcription (RT) has been used. Recently, a dual priming oligonucleotide (DPO) system for PERV detection has been developed [27]. Compared to the single PCR method, DPO provides a better detection rate, something that is very important in revealing the presence of PERV-C [27]. Recently, the use of a more sensitive method, real-time PCR, has been suggested for PERV-C detection [17,26]. Accurate PERV-C screening should include PCR assays with different primer pairs based on the sequence of the new variants of PERV-C [28]. This approach avoids the problem of false negative results, therefore increasing the safety of xenotransplantation. The level of PERVs depends of the kind of tissue. Measurement by real-time PCR of the *gag* fragment showed the highest level of PERV DNA in kidney samples, with lower levels detected in heart, liver and peripheral blood samples of domestic pigs [29]. In some of these cases, long PCR revealed partially deleted PERV DNA, indicating the lack of PERV complete genomes. Similarly, an analysis of *gag*, *pol* and *env* genes in Chinese Banna inbred minipigs revealed differences in PERV copy numbers in different tissues [30]. Screening of the source pig herd for PERV-C should include an analysis of both DNA and RNA. Despite the use of such a sensitive method as real-time PCR, in one study, it failed to detect PERV-C, whereas the subtype was detected when the test was proceeded with a reverse transcription step [16].

Another essential facet of xenotransplantation is selection of pigs that exhibit low-level expression of PERV-A and PERV-B. This is crucial for lowering the PERV infection risk. Therefore, not only should PERV proviruses be evaluated but also their expression in the tissues used for xenotransplantation. Measurement of PERV-A full-length mRNA expression (coding for Gag and Pol) using real-time RT-PCR revealed the highest level in the lung, spleen and lymph nodes and the lowest level in the pancreas [31]. In the same study, the expression of PERV at the protein level was confirmed with immunohistochemistry, with the signal found in the lungs, spleen and lymph nodes. PERV detection in 10-, 40-, 70- and 110 day-old Duroc