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COMMENTARY

WILEY Xenotransplantation

PERV inactivation is necessary to guarantee absence of pig-to-patient PERVs transmission in xenotransplantation

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In this issue of xenotransplantation, Scobie et al. commented on our recent production of PERV-inactivated pigs using CRISPR-cas9.[1] Based on the data of our studies, we believe there is an unneglectable risk of pig-to-human PERV transmission. We would like to provide some remarks and share our opinions about this issue here.

In a previous commentary on xenotransplantation, Joachim Denner has warned that PERV is the main microbiological risk for

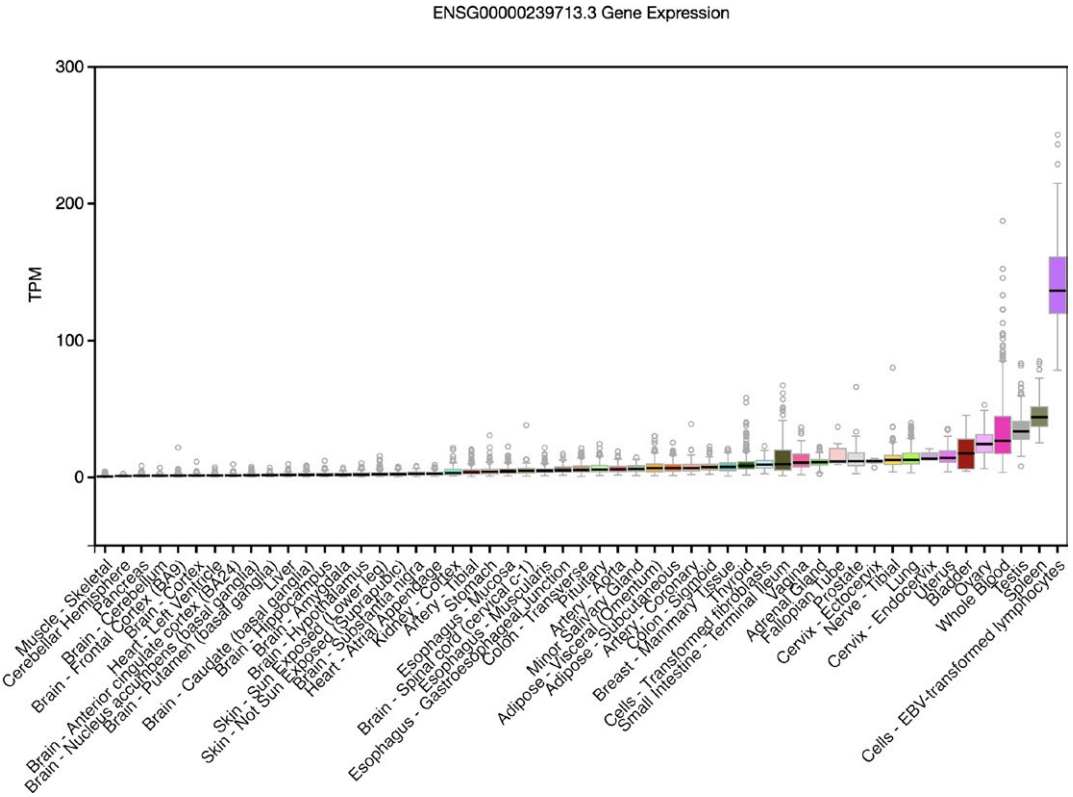

FIGURE 1 APOBEC3G expression level in multiple tissues from www.gtexportal.org

Güell, Niu and Kan contributed equally to this work

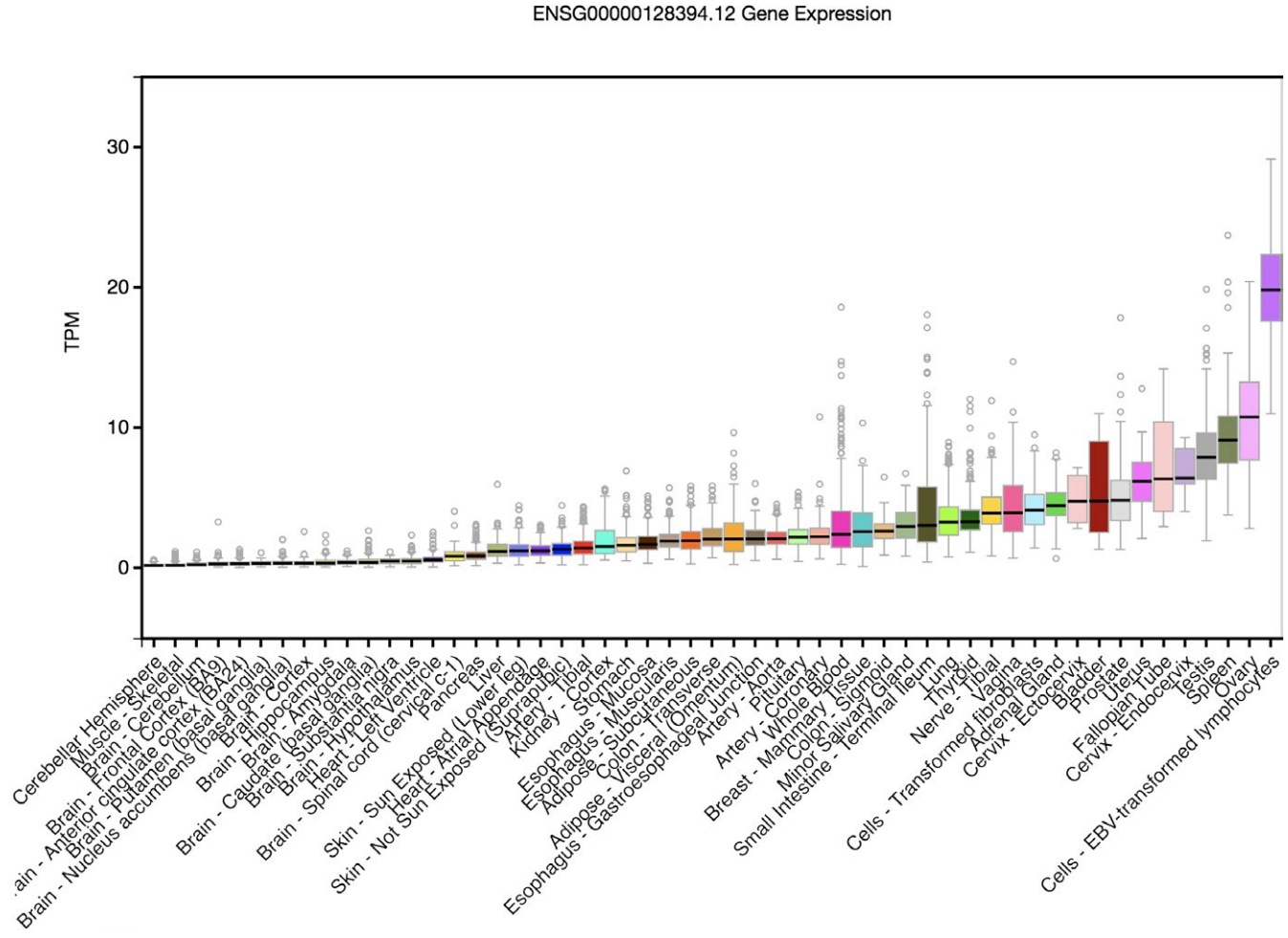

FIGURE 2 APOBEC3F expression level in multiple tissues from www.gtexportal.org

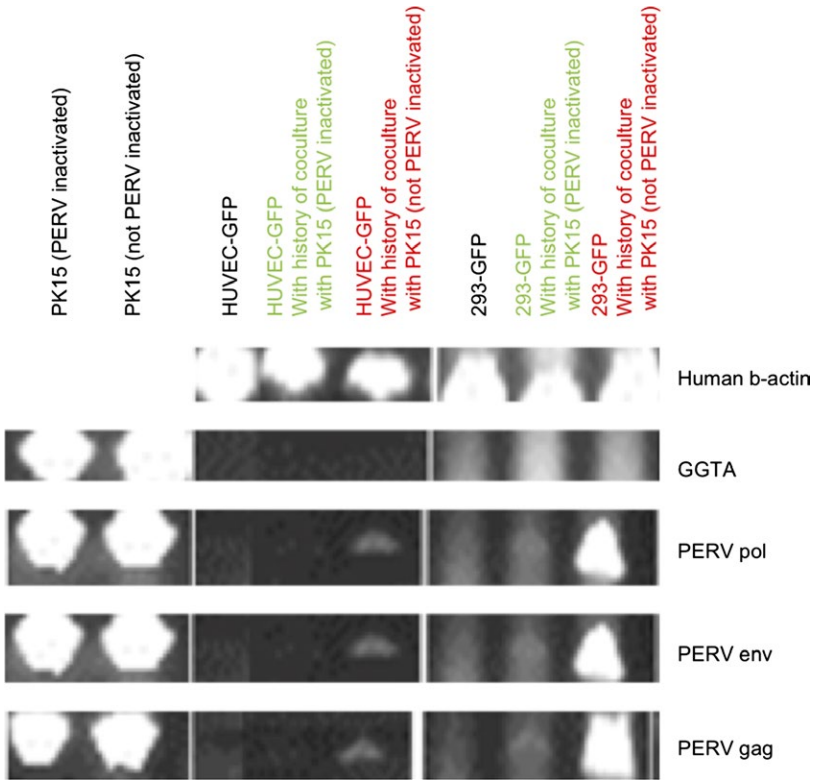

FIGURE 3 Internal data showing PERVs infection to human primary cells such as HUVEC

xenotransplantation.[2] We cannot agree more with this statement. If there is any technology that can eliminate PERVs, it should be exploited to protect patients. We and others detected clear and robust in vitro pig-to-human and human-to-human PERVs transmission.[1,3,4] Scobie et al. commented that our co-culture model using human 293 cells for PERVs infection is rather artificial due to the absence of restriction factor, APOBEC, in this human cell type. We think that the 293 cell model is an informative model despite absence of restriction factors. First, the absence of restriction factors does not reduce the clinical significance of the model. Multiple human primary tissues such as kidney, brain, muscle or liver are also negative or low for restriction factor, APOBEC (see Figure 1 and Figure 2). Second, presence of APOBEC reduces infection risk but does not eliminate it.[5] Infection of PBMCs and T cells which are high in APOBEC has been demonstrated.[6,7] Infection of PBMCs is specially alarming. This type of host cells would circulate over the xenograft and potentially spread infection to the rest of the host tissues. Infection of PBMCs seems to be facilitated by adaptation of PERVs after they infect an intermediary human cell with less antiviral factors such as 293 cells.[8] Multiple human cell types, such as artery and fibroblasts, with lower APOBEC (see Figure 1 and Figure 2) may contribute to PERV adaptation. It has been demonstrated that PERV can be transmitted from porcine cells to human endothelial cells, vascular fibroblasts and mesangial cells.[8] Third, we have also internally extended the 293 cell co-culture model to other cell types and observed same results as detected in 293 cells (see Figure 3 for infection of HUVEC cells by pig cells). HUVEC cells can produce high level of APOBEC upon viral infection[9] or upon interferon signaling[10] but still PERV infection occurs.

Scobie et al. also mentioned that it may be challenging to demonstrate the absence of infectivity for PERV-inactivated pigs as some pigs are claimed devoid of human tropic viruses. As a response of this statement, we have validated the complete elimination of PERV by multiple levels of assay. First, on the genomic level, we validated a fully inactivated PERV pol genotype. Second, on the protein level, we detected the elimination of functional pol activities via reverse transcriptase (RT) activity assay[1] (Figure S9 in Niu et al., *Science* 2017[1]). Lastly, we performed the infectivity assay and confirmed no pig-to-human PERV transmission for PERV-inactivated PK15, but robust PERV infection for WT PK15[11] (Figure 3C, D in Yang et al., *Science* 2015[11]). We believe that the combination of genotype confirmation, functional RT assay and infectivity test are sufficient to confirm the absence of PERV infection for PERV-inactivated pigs.

In the same commentary cited above, Joachim Denner mentioned that current experiences of clinical xenotransplantation only include transplantation of cells and tissues under no immunosuppression condition.[2] We would like to point out that most pathogen transmission reports were performed on encapsulated tissues with very limited information on xenograft viability to assess how many pig cells survived in the human host for short term and long term.[12,13] There is a lack of clinical data of xenotransplantation with solid vascularized organ transplantation into immune compromised patients to support the conclusion that there is no in vivo PERV transmission.

Genome-wide PERVs-inactivated pig is the only current option to guarantee the absence of pig-to-human PERVs transmission in xenotransplantation. PERV knock-down by siRNA[14] and PERV-C free strains cannot eliminate the risk of PERV transmission. siRNA reduces PERVs expression but it does not completely eliminate the presence of active PERVs.[14] Usage of PERV-C free pigs to avoid the generation of high replication recombinants PERV-A/C[15] is not a safe solution either. PERV-A and PERV-B have a well proven human tropism.[3] Pig-to-human and human-to-human PERV transmission have been demonstrated multiple times.[1,3,4] Recipients of xenografts will likely be under immunosuppressive regimes; therefore, maximum caution on PERV transmission should be taken.

We feel sympathy for the millions of patients waiting for organs, and we are trying our best to deliver safe and effective organs to those patients. We have made remarkably fast progress in addressing PERV-related safety issues within short time frames. We strive to provide our PERV-inactivated pig as a platform to the community for further genetic modifications to address other issues such as immunology, and we hope this could eventually solve the problem of human organ shortage for transplantation.

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REFERENCES

1. Niu D, Wei HJ, Lin L, et al. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. *Science*. 2017;357:1303-1307.
2. Denner J. Elimination of porcine endogenous retroviruses from pig cells. *Xenotransplantation*. 2015;22:411-412.
3. Wilson CA, Wong S, VanBrocklin M, Federspiel MJ. Extended analysis of the in vitro tropism of porcine endogenous retrovirus. *J Virol*. 2000;74:49-56.
4. Denner J, Specke V, Thiesen U, Karlas A, Kurth R. Genetic alterations of the long terminal repeat of an ecotropic porcine endogenous retrovirus during passage in human cells. *Virology*. 2003;314:125-133.
5. Jónsson SR, LaRue RS, Stenglein MD, Fahrenkrug SC, Andrésdóttir V, Harris RS. The restriction of zoonotic PERV transmission by human APOBEC3G. *PLoS ONE*. 2001;2:e893.
6. Specke V, Rubant S, Denner J. Productive Infection of Human Primary Cells and Cell Lines with Porcine Endogenous Retroviruses. *Virology*. 2001;285:177-180.
7. Denner J. Porcine endogenous retrovirus infection of human peripheral blood mononuclear cells. *Xenotransplantation*. 2015;22:151-152.
8. Martin U, Winkler ME, Id M, et al. Productive infection of primary human endothelial cells by pig endogenous retrovirus (PERV). *Xenotransplantation*. 2000;7:138-142.
9. Pauli EK, Schmolke M, Hofmann H, et al. High level expression of the anti-retroviral protein APOBEC3G is induced by influenza A virus but does not confer antiviral activity. *Retrovirology*. 2009;6:38.

10. Indraccolo S, Pfeffer U, Minuzzo S, et al. Identification of genes selectively regulated by IFNs in endothelial cells. *J Immunol*. 2007;178:1122–1135.
11. Yang L, Güell M, Niu D, et al. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science*. 2015;350:1101-1104.
12. Wynyard S, Nathu D, Garkavenko O, Denner J, Elliott R. Microbiological safety of the first clinical pig islet xenotransplantation trial in New Zealand. *Xenotransplantation*. 2014;21:309-323.
13. Gazda LS, Collins J, Lovatt A, et al. A comprehensive microbiological safety approach for agarose encapsulated porcine islets intended for clinical trials. *Xenotransplantation*. 2016;23:444-463.
14. Semaan M, Kaulitz D, Petersen B, Niemann H, Denner J. Long-term effects of PERV-specific RNA interference in transgenic pigs. *Xenotransplantation*. 2012;19:112-121.
15. Denner J. Recombinant porcine endogenous retroviruses (PERV-A/C): a new risk for xenotransplantation? *Arch Virol*. 2008;153:1421-1426.

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