Original Article

Differential Expression of Tripartite Motif-Containing Family in Normal Human Dermal Fibroblasts in Response to Porcine Endogenous Retrovirus Infection

(porcine endogenous retrovirus / tripartite motif-containing family / lipopolysaccharide / xenotransplantation / oligonucleotide microarray)

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Abstract. Antiretroviral restriction factors may play an essential role in the safety of xenotransplantation. Therefore, the present study focused on investigation of the changes in the tripartite motif-containing family (*TRIM*) gene expression in normal human dermal fibroblasts with and without lipopolysaccharide stimulation in response to porcine endogenous retrovirus infection. Analysis of the expression profile of *TRIMs* was performed using oligonucleotide microarrays and QRT-PCR. Nine (*TRIM1, TRIM2, TRIM5, TRIM14, TRIM16, TRIM18, TRIM22, TRIM27* and *TRIM31*) statistically significantly differentially expressed genes were found ($P < 0.05$, one-way ANOVA). In conclusion, comprehensive analysis of retroviral restriction factor gene expression in human dermal fibroblasts before and after porcine endogenous retrovirus infection with and without LPS stimulation may suggest association of the selected TRIMs with antiretroviral activity.

Introduction

Xenotransplantation can offer a potential solution to the shortage of allogeneic human organs, tissues and cells. However, the possibility of pathogen and even non-pathogenic microorganism interspecies transmission from xenografts to humans should also be considered (Denner and Tönjes, 2012). The possibility of spontaneous recombination between exogenous and endogenous viral sequences of human and animal viruses, which may lead to emergence of new pathogens (Denner, 2008; Lee et al., 2008), cannot be excluded, either.

In most cases, the risk of viral infections can be eliminated by selection and breeding of pathogen-free animals. This strategy is not suitable in the case of endogenous retroviruses such as porcine endogenous retroviruses (PERVs) (Scobie and Takeuchi, 2009; Denner and Tönjes, 2012). Replication of the retrovirus may depend on the balance between cellular cofactors and antiviral restriction factors (Mous et al., 2012). Antiviral restriction systems include e.g. the tripartite motif-containing (TRIM) family, apolipoprotein B mRNA-editing catalytic polypeptides (APOBEC), bone marrow stromal cell antigen 2 (BST-2, tetherin) or zinc finger antiviral protein (ZAP). Moreover, the TRIM protein family consists of over 70 members, which are also involved in different cellular processes, including proliferation, differentiation, development, oncogenesis or apoptosis besides antiviral restriction mechanisms (Meije et al., 2010; Laguette and Benkirane, 2012).

Lipopolysaccharide (LPS) is the most biologically active component of Gram-negative bacteria, which is responsible for the pathophysiological effects associated with infection and is recognized by Toll-like receptor 4 (TLR4) (Lu et al., 2008). In turn, TLR 4 stimulation can lead to production of interferons (IFNs) (Wang et al., 2008), which may be the key cytokines that mediate antiviral responses. Additionally, *TRIMs* are IFN-inducible genes (Uematsu and Akira, 2007; Uchil et al., 2013).

Previous studies have shown that antiviral restriction factors may play an essential role in the safety of xenotransplantation (Meije et al., 2010). Many attempts have been made to determine retrovirus interference by anti-

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Abbreviations: FBS – foetal bovine serum, HIV – human immunodeficiency virus, IFNs – interferons, LPS – lipopolysaccharide, NHDF – normal human dermal fibroblasts, PERV – porcine endogenous retrovirus, TLR4 – Toll-like receptor 4, TRIM – tripartite motif-containing family, ZAP – zinc finger antiviral protein.

viral proteins (Nisole et al., 2005; Perez-Caballero et al., 2008; Uchil et al., 2008; Meije et al., 2010), but it is still unclear whether PERVs may be inhibited by these factors (Abudu et al., 2006; Jónsson et al., 2007).

The differences in the expression patterns of *TRIM* family genes in human cells infected with PERVs have not been described so far. Therefore, the present study focused on investigation of the changes in the *TRIM* family gene expression in normal human dermal fibroblasts in response to porcine endogenous retrovirus infection.

Material and Methods

Cell culture conditions

Normal human dermal fibroblasts (NHDF cell line) were obtained from Clonetics (CC-2511; San Diego, CA) and routinely maintained in the FBM medium (Fibroblast Basal Medium, Lonza, Basel, Switzerland), supplemented with human fibroblast growth factor-basic (hFGF-B), insulin and gentamicin (FGM™ SingleQuots™; Lonza) at 37 °C in a 5% CO_2 incubator (Direct Heat CO2, Thermo Scientific, Waltham, MA).

Normal porcine kidney epithelial cells (PK15 cell line) were obtained from the American Type Culture Collection (ATCC CCL-33) and routinely maintained in the DMEM medium (Dulbecco's Modified Eagle Medium; Lonza), supplemented with 10% foetal bovine serum (FBS, Lonza) and gentamicin 25 mg/100 ml (Lonza) at 37 °C in a 5% CO_2 incubator (Direct Heat CO2, Thermo Scientific).

Both the cell number and viability were monitored by cell counting in the Bürker chamber after staining with 0.2% trypan blue (Biological Industries, Beit HaEmek, Israel). The experiment was performed with cells in the logarithmic phase of growth under the condition of ≥ 98% viability assessed by trypan blue exclusion.

LPS stimulation of NHDF cells, cytotoxicity test and PERV transmission assay by co-culture of NHDF and PK15 cells were performed as described previously by Kimsa et al. (2013).

Ribonucleic acid extraction

Total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. RNA extracts were treated with DNase I (RNeasy Mini Kit, Qiagen, Valencia, CA) according to the manufacturer's instructions. The quality of extracts was checked electrophoretically using 0.9% agarose gel stained with ethidium bromide (Sigma-Aldrich, St. Louis, MO). The results were analysed and recorded using the 1D Bas-Sys gel documentation system (Biotech-Fisher, Perth, Australia). Nucleic acid concentration was determined using a GeneQuant II RNA/DNA spectrophotometer (Pharmacia Biotech, Cambridge, UK).

Real-time QRT-PCR assay

PERV infectivity assay and detection of the copy number of human glyceraldehyde-3-phosphate dehydrogenase (*hGAPDH*) mRNA were performed as described previously (Cyganek-Niemiec et al., 2012; Kimsa et al., 2013). Oligonucleotide primers specific for *TRIM16* and *TRIM22* were designed on the basis of reference sequences (GenBank accession No. NM_006470 and NM_006074, respectively) using Primer ExpressTM Version 2.0 software (PE Applied Biosystems, Foster City, CA) (Table 1).

The analysis was performed using an Opticon™ DNA Engine Continuous Fluorescence Detector (MJ Research, Watertown, MA) and SYBR Green I chemistry (SYBR Green QuantiTect RT–PCR Kit; Qiagen). All samples were tested in triplicate. *GAPDH* was included as an endogenous positive control of amplification and integrity of extracts.

The thermal profile for one-step QRT-PCR was as follows: reverse transcription at 50 °C for 30 min, polymerase activation at 95 °C for 15 min, and then 30 cycles consisting of the following temperatures and time intervals: 94 °C for 30s, 65 °C for 45 s and 72°C for 40 s.

The point at which a PCR product is first detected above a fixed threshold, termed a cycle threshold (Ct), was determined for each sample. To quantify the results obtained by RT-PCR, a standard curve method was used, described previously by Strzalka-Mrozik et al. (2010). PCR products were separated in 6% polyacrylamide gels and visualized with silver salts.

The obtained results of mRNA copy number of the studied genes were recalculated per µg of total RNA.

Oligonucleotide microarray analysis

The oligonucleotide microarray analysis was performed in 12 samples: three samples of untreated and uninfected NHDF cells (NHDF); three samples of LPS-treated and uninfected NHDF cells (NHDF/LPS); three

Table 1. Characteristics of primers used for real-time QRT-PCR

Gene	Sequence of primers	Length of amplicon (bp)[a]	Tm (°C)[b]
TRIM16	Forward: 5'-TGCCGTTGTTCAGCGCAAATATTG- 3' Reverse: 5'- TGGTGTTGGTGACCTTGCGGTTC- 3'	152	82.0
TRIM22	Forward: 5'-CGACCTAATCGGCATCTGGCCA- 3' Reverse: 5'- CCTCGTTTATGCGGAATGTTTGGTG- 3'	202	80.0
hGAPDH	Forward: 5'-GAAGGTGAAGGTCGGAGTC-3' Reverse: 5'-GAAGATGGTGATGGGATTC-3'	226	80.1

[a]bp – base pairs; [b]Tm – melting temperature

samples of untreated and PERV-infected NHDF cells (NHDF/PK15) and three samples of LPS-treated and PERV-infected NHDF cells (NHDF/LPS/PK15). Total RNA was reversely transcribed and then the synthesis of biotinylated aRNA was performed with the use of GeneChip 3' IVT Express Kit (Affymetrix, Santa Clara, CA). aRNA was fragmented with the use of GeneChip 3' IVT Express Kit (Affymetrix) and hybridized with HG-U133A 2.0 (Affymetrix). Staining with streptavidin-FITC was performed according to the manufacturer's instructions (Affymetrix). Fluorescence intensity was measured with the use of Gene Chip Scanner 3000 7G and GeneChip® Command Console® Software (Affymetrix).

Statistical analyses

Statistical analyses were performed using Statistica 9.0 software (StatSoft, Tulsa, OK), and the level of significance was set at $P < 0.05$. Values obtained by the PCR technique were expressed as means and standard deviation (SD). One-way ANOVA and Tukey post hoc test were applied to evaluation of the differences in the expression of examined genes between PERV-infected and uninfected cells, untreated cells and cells treated with LPS.

Microarray data analysis was performed with the use of GeneSpring 12.0 platform (Agilent Technologies UK Limited, South Queensferry, UK). *TRIM* family transcripts were selected from the NetAffx Analysis Center database of Affymerix (http://www.affymetrix.com/analysis/index.affx). The normalized microarray data were used to compile a list of selected retroviral restriction factor genes whose expression appeared to be up- or down-regulated by a cut-off of at least 1.1-fold change. One-way ANOVA and Tukey post hoc test were applied to detection of differentially expressed genes at $P < 0.05$.

Results

Differential expression of viral restriction factor genes

In order to identify the *TRIM* protein family genes that could interfere with PERV infection, RNA from NHDF cells after stimulation with LPS and PERV infection was used to assess the gene expression profile using the HG-U133A 2.0 oligonucleotide microarrays, which enable analysis of 22,277 probe sets. These probe sets correspond to more than 18,400 transcripts and 14,500 well-characterized human genes. The expression of tripartite motif-containing genes was compared between the following groups: 1 – untreated and uninfected NHDF cells (NHDF), 2 – LPS-treated and uninfected NHDF cells (NHDF/LPS), 3 – untreated and PERV-infected NHDF cells (NHDF/PK15), 4 – LPS-treated and PERV-infected NHDF cells (NHDF/LPS/PK15).

Typing of differentially expressed genes was performed in a panel of 84 selected transcripts of 44 genes encoding the tripartite motif-containing protein family. Nine statistically significantly differentially expressed genes were found ($P < 0.05$, one-way ANOVA) (Table 2).

When the NHDF/PK15 samples were compared to NHDF, five statistically significantly differentially ex-

Table 2. Characteristics of tripartite motif-containing genes that exhibit differential expression in PERV-infected NHDF cells with and without LPS stimulation versus controls

Probe set	Gene symbol	Gene name	FC[a]					P value
			NHDF/PK15 vs. NHDF	NHDF/LPS/PK15 vs. NHDF	NHDF/LPS/PK15 vs. NHDF/PK15	NHDF/LPS/PK15 vs. NHDF/LPS	NHDF/LPS vs. NHDF	
208384_s_at	*TRIM1*	tripartite motif-containing 1	1.32↑[b]	1.17↑	1.13↓	1.04↑	1.13↑	0.0483
202342_s_at	*TRIM2*	tripartite motif-containing 2	1.08↑	1.23↑	1.26↑	1.67↑	1.11↑	0.0195
210705_s_at	*TRIM5*	tripartite motif-containing 5	1.03↑	1.31↑	1.30↑	1.40↑	1.07↓	0.0447
203148_s_at	*TRIM14*	tripartite motif-containing 14	1.68↑	3.62↑	2.15↑	1.74↑	2.08↑	0.0120
204341_at	*TRIM16*	tripartite motif-containing 16	4.40↑	4.79↑	1.09↑	1.07↓	5.13↑	< 0.0001
203637_s_at	*TRIM18*	tripartite motif-containing 18	1.16↑	1.59↑	1.37↑	1.84↑	1.16↓	0.0184
213293_s_at	*TRIM22*	tripartite motif-containing 22	1.17↓	1.08↓	1.09↑	1.36↑	1.47↓	0.0309
212118_at	*TRIM27*	tripartite motif-containing 27	1.31↓	1.24↓	1.06↑	1.09↑	1.36↓	0.0263
215444_s_at	*TRIM31*	tripartite motif-containing 31	1.05↓	1.30↓	1.24↓	1.12↓	1.16↓	0.0492

Statistical significance: *$P < 0.05$, one-way ANOVA

[a]FC – fold change

[b]↑, ↓ higher and lower expression in the PERV-infected NHDF cells (NHDF/PK15), and LPS-treated and PERV-infected NHDF cells (NHDF/LPS/PK15), and LPS-treated NHDF cells versus controls (NHDF)

pressed genes were found (P < 0.05, Tukey post hoc test). Up-regulated transcripts were recorded for three genes (*TRIM1*, *TRIM2*, *TRIM16*) and down-regulated transcripts were found for two genes (*TRIM22*, *TRIM27*). Four statistically significantly differentially expressed genes were detected when NHDF/LPS/PK15 samples were compared to the NHDF ones (P < 0.05, Tukey post hoc test). Among these genes, three (*TRIM14*, *TRIM16*, *TRIM18*) were up-regulated, whereas one (*TRIM27*) was down-regulated. In the NHDF/LPS/PK15 samples, when compared to the NHDF/PK15 samples, two (*TRIM14, TRIM18*) statistically significantly differentially expressed genes (P < 0.05, Tukey post hoc test) were up-regulated. In the NHDF/LPS/PK15 samples, when compared to the NHDF/LPS samples, four (*TRIM2*, *TRIM5*, *TRIM14*, *TRIM18*) statistically significantly differentially expressed genes (P < 0.05, Tukey post hoc test) were up-regulated. In the NHDF/LPS samples, when compared to the NHDF ones, three statistically significantly differentially expressed genes were observed (P < 0.05, Tukey post hoc test). Among these genes, one (*TRIM16*) was up-regulated and two (*TRIM22*, *TRIM27*) were down-regulated.

Validation of microarray data by QRT-PCR

The expression levels of *TRIM16*, which had the highest fold change in microarray analysis, and *TRIM22*, which was previously reported as antiviral restriction factor, were analysed by QRT-PCR. The mRNA levels of *TRIM16* and *TRIM22* were detected in all studied groups (Fig. 1A, B). Comparative analysis of *TRIM16* mRNA copy number per 1 μg RNA revealed statistically significant differences between the NHDF/PK15 (39205 ± 12140 copy number/μg total RNA), NHDF/LPS/PK15 (31411 ± 15732 copy number/μg total RNA), NHDF/LPS (30803 ± 10550 copy number/μg total RNA) and NHDF (22713 ± 9708 copy number/μg total RNA) samples (P = 0.0482, one-way ANOVA test). The quantitative relations between all studied groups showed statistically significant differences between the NHDF/PK15 and NHDF samples (P = 0.0399, Tukey post hoc test). There was no statistically significant difference between the NHDF/LPS/PK15 and NHDF (P = 0.9485, Tukey post hoc test) samples, NHDF/PK15/LPS and NHDF/PK15 (P = 0.1236, Tukey post hoc test) samples, NHDF/PK15/LPS and NHDF/LPS (P = 0.8359, Tukey post hoc test) samples, and NHDF/LPS and NHDF (P = 0.5395, Tukey post hoc test) samples.

Statistically significant differences of the *TRIM22* mRNA level between NHDF/PK15 (120247 ± 20614 copy number/μg total RNA), NHDF/LPS/PK15 (132493 ± 30210 copy number/μg total RNA), NHDF/LPS (122043 ± 5983 copy number/μg total RNA) and NHDF (176169 ± 9361 copy number/μg total RNA) samples were also observed (P = 0.0408, one-way ANOVA test). Corresponding to the results obtained for the copy number of *TRM16*, there was a statistically significant difference between the NHDF/PK15 and NHDF samples (P = 0.0462, Tukey post hoc test). There was no statistically significant difference between the following groups: NHDF/LPS/PK15 and NHDF (P = 0.1211, Tukey post hoc test), NHDF/PK15/LPS and NHDF/PK15 (P = 0.8787, Tukey post hoc test), NHDF//PK15/LPS and NHDF/LPS (P = 0.9398, Tukey post hoc test), and NHDF/LPS and NHDF (P = 0.0832, Tukey post hoc test).

The tendency of both *TRIM* mRNA levels to be up- or down-regulated was consistent with the microarray data.

Discussion

Numerous studies have demonstrated that porcine endogenous retroviruses can be transmitted to different human cell lines *in vitro* (Specke et al., 2001; Li et al., 2006), including dermal fibroblasts which can be ex-

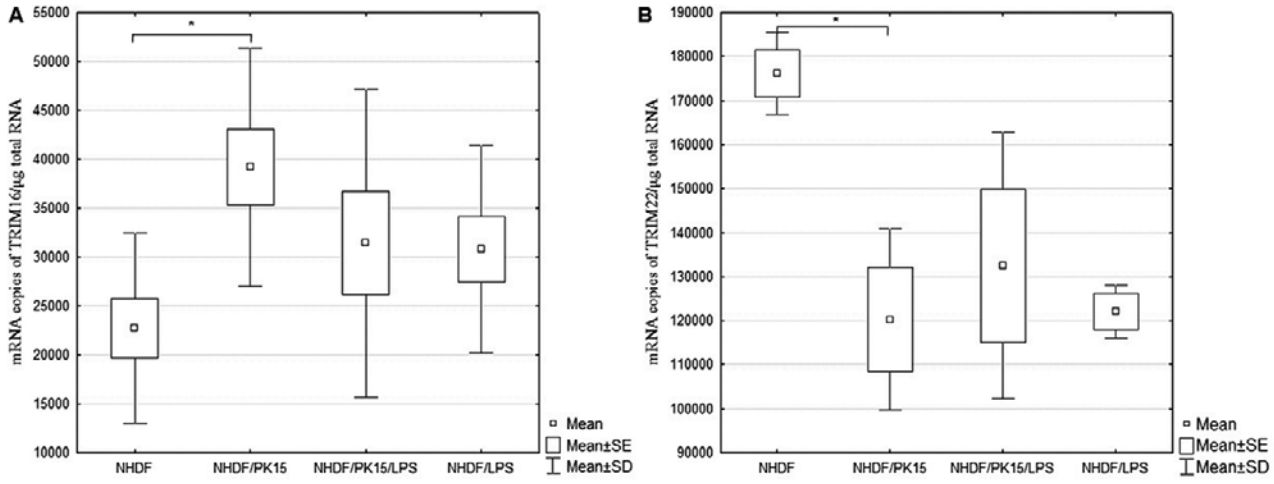

Fig. 1. The mRNA level of *TRIM16* (**A**) and *TRIM22* (**B**) in the control (NHDF), LPS-treated (NHDF/LPS) and PERV-infected NHDF cells with (NHDF/LPS/PK15) and without (NHDF/PK15) LPS stimulation.
The bars represent means ± standard deviations (SD) of copy numbers per 1 μg of total RNA; statistical significance: *P < 0.05, Tukey post hoc test

posed to PERVs by the use of pig skin as a temporary biological dressing (Kimsa et al., 2013; Scobie et al., 2013).

The possibility of interspecies virus transmission has affected investigations of antiviral strategies, aiming to reduce the risk of virus infection (Scobie and Takeuchi, 2009). These strategies include selection and breeding of pathogen-free animals, antiretroviral therapy (Shi et al., 2007) or use of an RNA interference mechanism (Dieckhoff et al., 2008; Ramsoondar et al., 2009; Semaan et al., 2012). Moreover, the ability of viruses to replicate is also dependent on multiple interactions between the virus and the target cell. Antiretroviral intrinsic immunity factors are cell type-specific, and viruses that are unable to counter these intrinsic immunity factors in a cell are blocked in their replication (Pineda et al., 2007).

IFNs are considered to be the first line of defence against viral infection. Kornbluth et al. (1989) demonstrated that IFNs- and LPS-treated macrophages did not contain detectable viral RNA or DNA after human immunodeficiency virus (HIV) infection *in vitro*. In our previous studies we did not reveal PERV B DNA in untreated or LPS-treated NHDF cells. However, PERV A DNA, and PERV A and PERV B RNA were observed in both NHDF cultures (Kimsa et al., 2013).

Simard et al. (2008) showed that the inhibition of HIV-1 replication in human monocyte-derived macrophages (MDM) treated with LPS can result from the induction of type I INFs. Other authors explained that the CD14/TLR4 complex acting in response to LPS involves the MyD88-independent pathway that induces production of type I IFNs (Doyle et al., 2002; Simard et al., 2008). The Kornbluth's study (Kornbluth et al., 1989) also suggested that treatment with IFNs or LPS may prevent establishment of productive retrovirus infection. Similarly, our previous research revealed a statistically significant decrease of the copy number of PERV DNA in LPS-treated NHDF cells after PERV infection, suggesting the possibility that treatment with LPS may inhibit the porcine endogenous retrovirus infection. However, it is not clear whether LSP treatment was effective in inhibition of PERV because the copy number of the PERV A DNA decreased but that of PERV A RNA increased (Kimsa et al., 2013). Indeed, previously published data revealed that LPS can promote virus production in a monocytic cell line or it can strongly inhibit virus replication in primary human MDM cells (Kedzierska et al., 2003; Brenchley et al., 2006; Simard et al., 2008).

TRIM proteins are also believed to be efficient antiretroviral factors, and our research therefore focused on tripartite motif-containing family gene expression in NHDF fibroblasts with and without LPS stimulation in response to PERV infection. Nine statistically significantly differentially expressed *TRIM* genes were found: *TRIM1*, *TRIM2*, *TRIM5*, *TRIM14*, *TRIM16*, *TRIM18*, *TRIM22*, *TRIM27* and *TRIM31*. Among these, *TRIM1*, *TRIM2*, *TRIM5*, *TRIM14*, *TRIM16* were up-regulated and *TRIM18*, *TRIM22*, *TRIM27* were down-regulated in PERV-infected NHDF cells with and without LPS stimulation compared to the control NHDF cells. In the previously published studies, microarray analyses revealed altered gene expression of *TRIM*, but after INF stimulation (Martinez et al., 2006; Carthagena et al., 2009; Boulware et al., 2010). In MDM cells, type I IFN caused up-regulated expression of 16 *TRIM* genes (*TRIM5*, *6*, *14*, *19*, *20*, *21*, *22*, *25*, *26*, *31*, *34*, *35*, *38*, *56*, *58* and *69*) and down-regulation of five genes (*TRIM28*, *37*, *54*, *59* and *66*). Type II IFN only induced up-regulation of seven genes (*TRIM19*, *20*, *21*, *22*, *25*, *56* and *69*) and down-regulation of 11 genes (*TRIM2*, *4*, *9*, *16*, *16L*, *28*, *32*, *37*, *54*, *59* and *66*). In turn, in peripheral blood lymphocytes, 14 *TRIM* genes were up-regulated by type I IFN (*TRIM5*, *6*, *14*, *19*, *20*, *21*, *22*, *25*, *26*, *31*, *34*, *35*, *38* and *56*) and seven by type II IFN (*TRIM19*, *20*, *21*, *22*, *26*, *56* and *69*). Only one gene, *TRIM16*, was significantly down-regulated following IFN treatment (Carthagena et al., 2009). In turn, our research revealed a statistically significant increase of *TRIM16* expression after LPS stimulation, which may suggest its association with antiretroviral activity.

In the current study, among nine differentially expressed *TRIM* genes, four have previously been reported to display antiviral activity, including *TRIM1* (Yap et al., 2004; Rajsbaum et al., 2008), *TRIM5* (Asaoka et al., 2005; Sakuma et al., 2007; Carthagena et al., 2008), *TRIM22* (Tissot and Mechti, 1995; Barr et al., 2008) and *TRIM31* (Uchil et al., 2008).

TRIM5α-mediated restriction is species-specific and various viruses are blocked in different primate species. Primate TRIM5α proteins have distinct pre- and post-integration restriction activities against a wide range of retroviruses and lentiviruses. However, human TRIM5α potently restricts N-tropic murine leukaemia virus (N-MLV), equine infectious anaemia virus (EIAV) but not HIV-1 (Diaz-Griffero et al., 2006; Sakuma et al., 2007). Kaiser et al. (2007) reported that the human TRIM5 protein can be active against an ancestral form of endogenous gammaretroviruses (chimpanzee endogenous retrovirus 1, CERV1). TRIM5α can also play a role in LPS-triggered immune activation through the TLR4 pathway (Pertel et al., 2011).

TRIM22 plays a role in reduction of HIV-1 infection by binding to the viral gag protein and interfering with virus budding, and it is known to inhibit the activity of the HIV-1 LTR (Tissot and Mechti, 1995; Barr et al., 2008). Analysis of the *TRIM22* expression by microarray analysis and real-time PCR showed its over-expression in IFNα- and LPS-stimulated as well as in HIV-infected MDM and 293T cells (Bouazzaoui et al., 2006). In contrast, our research revealed inhibited *TRIM22* expression in PERV-infected and LPS-stimulated cells. Sewram et al. (2009) suggested that patients who express low levels of *TRIM* are more likely to become HIV-1 infected than those with high levels of expression, or that HIV-1 infection is associated with dysregulation of *TRIM* mRNA expression. In turn, Rold and

Aiken (2008) showed that the human TRIM protein was rapidly degraded by a proteasome-dependent mechanism upon encounter of a restriction-sensitive virus.

Our observation may also suggest a role in antiviral defence for the five additional *TRIMs*, *TRIM2*, *TRIM14*, *TRIM16*, *TRIM18* and *TRIM27*. Geiss et al. (2002) and Martinez et al. (2006) observed that *TRIM14, TRIM18* were up-regulated and *TRIM22*, *TRIM27* down-regulated in response to influenza virus infection in a human epithelial cell line and macrophages, respectively, which is partially consistent with our study. In contrast to our research, Rajsbaum et al. (2008) and Martinez et al. (2006) showed that expression of *TRIM16* was down-regulated in immune cells. An important function of the TRIM16 protein is mediating transfer of ubiquitin to the substrates, and therefore it is involved in a number of cellular processes such as cell signalling, survival pathways or differentiation (Bell et al., 2012). Boulware et al. (2010) also showed many down-regulated genes using oligonucleotide microarray analysis such as *APOBEC3A*, *APOBEC3G*, *TRIM5* and *TRIM22* in peripheral blood cells, but after ART (antiretroviral therapy). The differences may be due to the fact that constitutive expression and IFN-inducibility of *TRIM* genes are cell type dependent, which may have an impact on the antiviral properties of TRIMs (Carthagena et al., 2008; Rajsbaum et al., 2008).

There are many reports on different antiretroviral restriction factors acting after virus infection, including PERV infection (Delebecque et al., 2006; Jónsson et al., 2007; Dorrschuck et al., 2008; Lee et al., 2011a,b). However, microarray analysis of expression of all *TRIM* genes in response to porcine endogenous infection in human cells with and without LPS stimulation was not performed in previous studies.

Previous studies revealed that PERVs are insensitive to restriction by divergent mammalian TRIM5a proteins from human, African green monkey, rhesus macaque, squirrel monkey, rabbit or cattle. The strongest restriction was produced by human TRIM5a, but this led to only around a three-fold reduction in infectivity, indicating that it is unlikely to act as a barrier to PERV cross-species transmission. The reason for poor sensitivity of gammaretroviruses to TRIM5α may be due to the capsid structure (Wood et al., 2009). In turn, Lee et al. (2011a) revealed that human TRIM5α showed inhibitory activity against N-tropic MLV and N-tropic mutant PERV, but exhibited no antiviral activity against Moloney murine leukaemia virus or wild-type PERV. Retroviral capsid sequences are conserved within these viruses, and the PERV capsid region is highly similar to that of MLV. However, there are also many other human TRIM proteins that may restrict PERVs (Nisole et al., 2005). Likewise, the present study revealed altered expression of *TRIM1*, *TRIM2*, *TRIM5*, *TRIM14*, *TRIM16*, *TRIM18*, *TRIM22*, and *TRIM27* in response to PERV infection.

In conclusion, the comprehensive analysis of retroviral restriction factor gene expression in human dermal fibroblasts before and after PERV infection with and without LPS stimulation may suggest the association of selected TRIMs with antiretroviral activity. Unfortunately, a lack of experiments of over-expression or knockdown of these candidate genes to show their antiretroviral effect may be a limitation of our research. Therefore, there is a need to further study TRIMs and the related molecules to understand the host cell-retrovirus relationships, particularly those that may concern xenotransplantation.

Acknowledgement

The authors declare that there are no conflicts of interest.

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