Acta virologica **61**: 299 – 307, 2017 doi:10.4149/av_2017_308

Cross-species hybridization of foot-and-mouth disease virus-infected BHK-21 cells using human and mouse oligonucleotide microarrays

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Received August 8, 2016; revised December 19, 2016; accepted June 27, 2017

Summary. – Foot-and-mouth disease virus (FMDV) has a dual capacity to induce either acute or persistent infection in host animals. Establishment of an *in vitro* cell model of FMDV persistent infection facilitates the study of the mechanism underlying this type of infection. In this study, we analyzed gene expression profiles of both acute and persistent infections using cross-species microarrays. Our data suggest that human microarrays are more efficient than mouse microarrays in hybridization with cDNA from BHK-21 cells although the mouse is closer to the Syrian hamster in taxonomy. A set of differentially expressed genes (DEGs) that may be involved in the determination of acute or persistent infection was identified by using human or mouse microarrays. Seven common DEGs were found in both human and mouse arrays and showed similar fold changes. Among the DEGs, 33 genes were selected for further validation by using qRT-PCR and presented consistent results. The analysis of Gene Ontology Biological Processes indicated that various biosynthetic and metabolic processes were negatively regulated in the group of acute infection whereas multicellular organismal development processes were positively regulated in the group of persistent infection. Our study demonstrates the plausibility and utility of using cross-species microarrays to study FMDV-infected mammalian cells. The combined use of two types of microarrays can be more informative in exploring the mechanisms underlying the infections of FMDV.

Keywords: foot-and-mouth disease virus; BHK-21; microarray; cross-species hybridization; acute infection; persistent infection

Introduction

Foot-and-mouth disease virus (FMDV) is the type species of the *Aphthovirus* genus within the *Picornaviridae* family. Infection of FMDV causes a highly contagious and economically important disease in cloven-hoofed animals, including cattle, pigs, sheep and goats; this disease is characterized by vesicular lesions in the mouth and on the feet, teats and nares (Sen and Saha, 1994; Brown, 1999; Balamurugan *et al*., 2004; Grubman and Baxt, 2004; Arzt *et al*., 2011). FMDV has a dual capacity to induce either an acute infection in cloven-hooved animals, or an inapparent, persistent infection in ruminants (Brown, 1999; Grubman and Baxt, 2004; Mahy, 2005). FMDV usually becomes immediately cytopathic to the cultured cells, in which it can also establish a long-term persistent infection (de la Torre *et al*., 1985; Huang *et al*., 2011).

To facilitate the investigation of the mechanisms underlying FMDV persistence, an *in vitro* model of persistent infection was established with a biological clone of FMDV as well as cloned BHK-21 cells (Clarke, 1983; de la Torre *et al*., 1985, 1988; Cai *et al*., 2013). A remarkable feature of FMDV persistence in BHK-21 cells is the coevolution of cell-virus, in which the cells become gradually more resistant to FMDV, which in turn becomes more virulent to the

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Abbreviations: BHK-21 = Baby hamster kidney 21; CSH = cross-species hybridization; DEGs = differentially expressed genes; FMDV = foot-and-mouth disease virus; GO:BP = Gene Ontology Biological Processes; qPCR = quantitative PCR

parental BHK-21 cells (de la Torre *et al.*, 1988, 1989; Martin Hernandez *et al.*, 1994; Herrera *et al.*, 2008). Although genetic heterogeneity and phenotypic flexibility of FMDV in persistence have been well documented, it is recognized that it is the cell, instead of the virus, that plays a critical role in the initiation of persistence (Martin Hernandez *et al.*, 1994; Zhang *et al.*, 2013).

A microarray technique has been extensively used to study the changes in gene expression profiles in numerous cases. Unfortunately, the gene array of Syrian hamster is currently not available since the whole genome of Syrian hamster is unknown, which greatly hampers investigators to study the gene expression in the BHK-21 cells with FMDV persistence using a microarray technique. Recently, our lab first used the commercial human whole-genome oligo microarray on BHK-21 cell samples, and demonstrated the approach of cross-species hybridization (CSH) was actually feasible (Huang *et al.*, 2011; Zhang *et al.*, 2013). Plenty of information was derived from human microarray data (Zhang *et al.*, 2013) and some of the new information from the present study may be even more valuable in elucidating the mechanism underlying FMDV infection.

There have been some issues regarding the cross-species hybridization (CSH) approach (Nieto-Diaz *et al.*, 2007). CSH has been considered as a non-standard application of microarrays (Adjaye *et al.*, 2004). It is known that the probes used for most of arrays are designed according to the sequences from one species, therefore, these probes may not exactly match the target cDNA derived from other species at many different nucleotide positions (Renn *et al.*, 2004; Bar-Or *et al.*, 2006). To overcome the inherently low signal of CSH, comparative cross-species hybridization was adopted. In CSH, the performance of RNA isolated from the target species hamster was compared between two different reference species (human and mouse). This method had been taken by many researchers and the feasibility was also clarified (Rifkin *et al.*, 2003; Held *et al.*, 2004; Ji *et al.*, 2004; Nagpal *et al.*, 2004; Nuzhdin *et al.*, 2004; Saetre *et al.*, 2004; Brodsky *et al.*, 2005; Chalmers *et al.*, 2005; Gilad *et al.*, 2005; Nowrousian *et al.*, 2005; Neufeld *et al.*, 2006; Vallee *et al.*, 2006; Walker *et al.*, 2006).

Syrian hamster (*Mesocricetus auratus*) belongs to the same rodent family as mouse. It is postulated that genes in Syrian hamster, compared with human ones, might be more conserved with those in mouse. Thus, the use of mouse arrays may reveal more information related to changes in the gene expression in Baby hamster kidney (BHK-21) cells, compared to the use of human arrays. In the present study, we applied these two types of arrays to investigate the gene expression profiles in BHK-21 cells and to evaluate whether mouse arrays were able to provide more information than human ones. A combined use of two types of arrays was expected to be more informative than the use of either type alone, and can also be very helpful in providing more information regarding the mechanistic differences between acute and persistent infections of FMDV in the mammalian cells such as BHK-21.

Materials and Methods

Cells and viruses. Virus strains of serotype O FMDV were obtained from Lanzhou Veterinary Research Institute, Chinese Academy of Agriculture Sciences. BHK-21 cells were provided by China Center for Type Culture Collection (CCTCC). Persistently infected BHK-21 cell lines were provided by Dr. Huang. In acute infection, cells were infected with FMDV at MOI of 3×10^{-7} (lower titers of virus induced more stable response of infection). In persistent infection, persistently infected BHK-21 after 57 passages (PI57) were used for microarray and qRT-PCR analysis. Cells were cultured in Minimum Essential Medium (MEM, GiBCO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GiBCO, USA) at 37°C with 5% CO_2.

Virus infection and RNA extraction. Total RNA was extracted from acutely and persistently infected BHK-21 cells, respectively, using Trizol reagent (Life Technologies, Carlsbad, USA) according to the manufacturer's instructions. RNA was purified with RNeasy Mini Kit (Qiagen p/n 74104) and treated with baseline-ZERO DNase (EPICENTRE, Cat. No. DB0711K). The RNA quality was evaluated by agarose gel electrophoresis, and the quantity was determined by spectrophotometer (Eppendorf).

Microarray hybridization. One microgram of purified RNA was used for cDNA synthesis prior to hybridization to human or mouse whole-genome arrays (Agilent 4X44 K). The cDNA from uninfected BHK-21 cells was labeled with Cy3-dUTP, while the cDNA from FMDV acutely or persistently infected BHK-21 cells was labeled with Cy5-dUTP using the Agilent low RNA input linear amplification kit. Hybridization was performed overnight at 65°C with human or mouse whole-genome arrays (Agilent 4X44 K) using Agilent Gene Expression Hybridization Kit (Agilent p/n 5188-5242). Arrays were washed and scanned using an Agilent scanner (Agilent Technologies, Santa Clara, CA).

Data analysis. Agilent Feature Extraction software (version 11.0.1.1) was used to analyze array images and normalize the raw data with Lowess method. The data was subsequently processed using the GeneSpring GX v12.0 software package (Agilent Technologies). After data normalization, genes with the ratio of fluorescence signal intensity ≥1 (the value from virus-infected samples divided by the value from uninfected samples) were selected for further analysis. Differentially expressed genes were identified through fold change filtering. Gene functional clustering was conducted with DAVID online bioinformatics resources 6.7 (Huang da *et al.*, 2009a; Huang da *et al.*, 2009b).

Real-time quantitative RT-PCR (qRT-PCR). The reaction of reverse transcription for cDNA synthesis included the following components: 1 μg of total RNA, 1 μg of Oligo (dT)15 primers, 4 μl 5× RT

buffer, 2 µl 10 mmol/l dNTP mix, 40 U RNasin and 200 U moloney murine leukemia virus (MMLV) reverse transcriptase (Promega). Real-time quantitative PCR was performed using SYBR green dye (Invitrogen) and Premix Taq (Promega). Primers were designed by using software Primer Premier 5. The sequences of primers are listed in Table S4. A two-step PCR (15 s at 95°C and 60 s at 60°C) was performed with 40 cycles after an initial activation step (95°C for 10 min) using the CFX96TM real-time PCR detection system (BIO-RAD). The melting curves from 60°C to 90°C were used to generate the standard melting curve.

Results

An overall comparison was performed between human and mouse microarrays

Human and mouse arrays were applied to evaluate the gene expression profiles in the FMDV-infected (acutely and persistently) and uninfected BHK-21 cells (used for normalization). There were about 41,000 probes in both human and mouse microarrays. Each probe was a 60-mer oligonucleotide with appropriate sensitivity and specificity for hybridization.

MA Plot method was used to perform an overall evaluation on human and mouse two-channel microarrays. In each array, acute and persistent infections were also analyzed comparatively. There were obvious differences between human and mouse microarrays in terms of the signal intensity (Fig. 1). Surprisingly, there were more MA points in human arrays than those in mouse arrays, indicating that human arrays hybridized more cDNAs of Syrian hamster than mouse arrays. In human arrays, the signal intensity in persistent infection was stronger than that in acute infection (Fig. 1a), while in mouse arrays, the signal intensity in two types of infections was almost the same (Fig. 1b). These results indicated that mouse microarray was less informative than the human microarray in hybridization with cDNA from

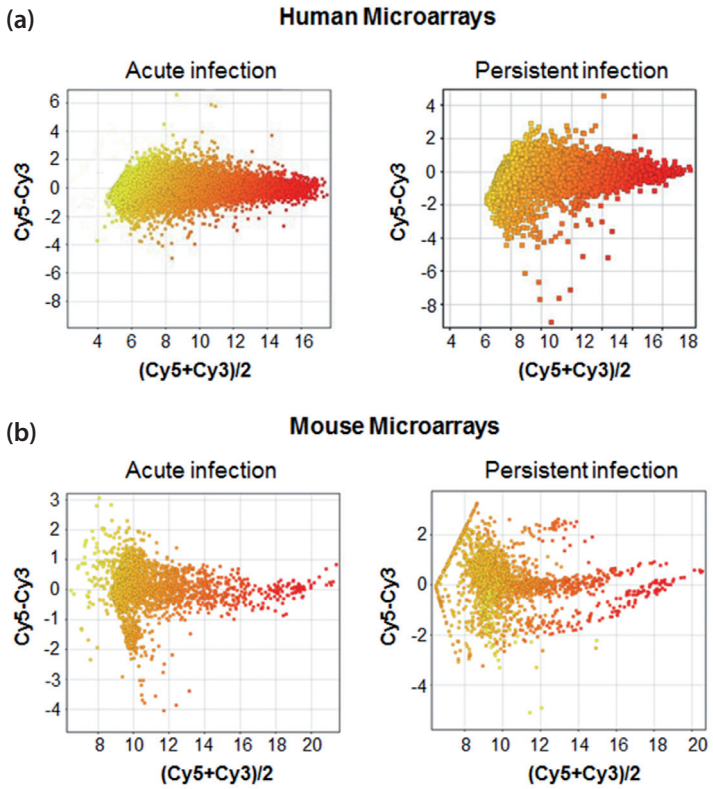

Fig. 1

Two-channel human and mouse microarrays were used to evaluate the signal intensity in acute and persistent infections

The MA Plot was used to assess the difference vs. the average of probes between two samples and also used in the two-color arrays to assess the relation between Cy3 (uninfected BHK-21 cell samples) and Cy5 (virus-infected BHK-21 cell samples) channels of the hybridization. The x-axis represents the average $\log_2$ ratio of two channels, while the y-axis represents the difference of $\log_2$ ratio between two channels. The left panels of Fig. 1a and 1b represent acute infection while the right panels represent persistent infection. **(a)** Signal intensity of human microarrays in both acute and persistent infection. **(b)** Signal intensity of mouse microarrays in both acute and persistent infection.

Table 1. Number of hybridized probes and differently expressed genes in human and mouse arrays

	Acute infection		Persistent infection	
	Human	Mouse	Human	Mouse
Detected genes	4618	1794	4618	1794
DEGs	370	269	506	483

A fold change filtering was performed to identify significant differentially expressed genes between the two samples. The $\log_2$ ratio of fold changes >= 1 or ≤ -1 was set as the threshold.

BHK-21 cells, although the mouse is closer to the Syrian hamster than human in taxonomy. These observations were consistent with previous studies, in which human and mouse microarrays were used to hybridize woodchuck samples (Wang *et al*., 2009).

Differentially expressed genes (DEGs) were thoroughly compared between human and mouse microarrays

The results from the human and mouse arrays were obtained from four samples in this study. In both groups of acute and persistent infections, human microarrays detected 4618 genes while mouse ones merely detected 1794 genes (Table 1 and Fig. 1), indicating that human microarrays were much more efficient than mouse microarrays in these conditions. However, there was no significant difference between human and mouse arrays in terms of the DEG numbers although human arrays also detected more DEGs than mouse arrays in these two types of infections (Table 1).

We then analyzed fold change ($\log_2$ ratio) distribution in DEGs. Human and mouse microarrays had similar distribution trends in both acute and persistent infections (Fig. 2). The fold changes ($\log_2$ ratio) of most of DEGs in both human and mouse arrays ranged from -2 to 2 (Fig. 2). In the range from -10 to -2, both human and mouse microarrays detected 84 DEGs in persistent infection, whereas in the acute infection, the human arrays detected 19 DEGs while the mouse ones merely detected 9 DEGs. In the range from 2 to 10, human arrays detected 11 DEGs in acute infection and 17 DEGs in persistent infection, while mouse arrays detected 25 DEGs in acute infection and 37 DEGs in persistent infection, indicating that mouse arrays were able to identify more differentially expressed genes than human arrays although they detected fewer genes in total.

Significant DEGs were found in human and mouse arrays

It is generally recognized that genes with large fold-change are of great interest to investigators. Fold changes ≥ 4 or ≤ 0.25 were defined as the threshold for significant DEGs in this study. Some of the most significant DEGs found in human and mouse microarrays were summarized in Table S1 and Table S2.

In the group of acute infection, many classes of genes were identified in both human and mouse arrays, including protein phosphorylation genes such as PIK3R4 (0.01-fold, Table S1) and LRRK1 (0.20-fold, Table S2), translation-related genes such as RPL37 (4.44-fold, Table S1) and WBSCR22 (0.14-fold, Table S2), and transcription-related genes such as DEDD (6.77-fold, Table S1) and ERC1 (14.42-fold, Table S2). Notably, some unique classes of genes, including ubiquitin cycle-related genes such as RNF139 (0.18-fold, Table S1) and KPTN (4.63-fold, Table S1), and virus-response genes such

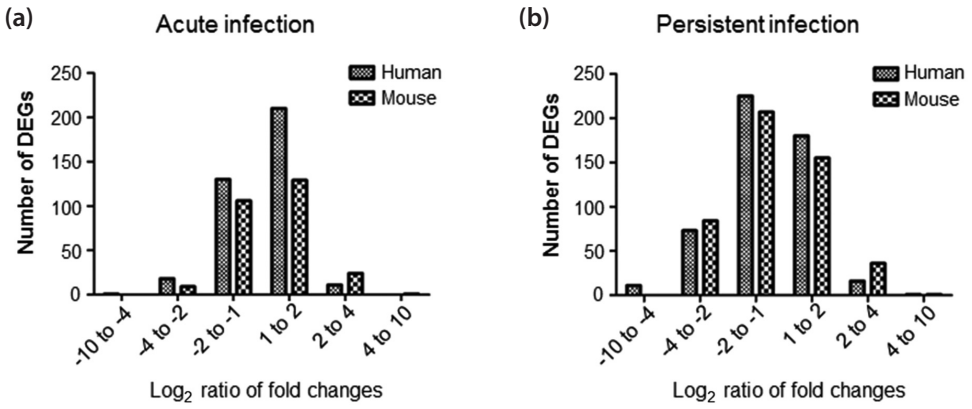

Fig. 2

Differentially expressed genes (DEGs) with distinct fold changes were identified by using human and mouse microarrays in both acute and persistent infections

(a) The comparison of DEGs' distribution between human and mouse microarrays in the condition of acute infection. **(b)** The comparison of DEGs' distribution between human and mouse microarrays in the condition of persistent infection. The fold changes are presented in a $\log_2$ ratio (x-axis).

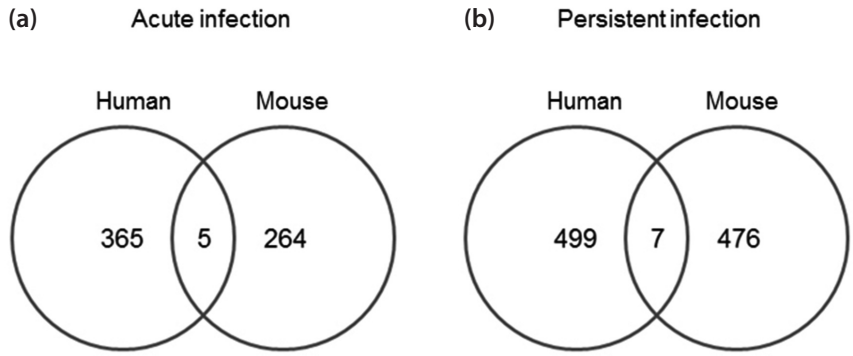

Fig. 3

Co-DEGs were identified in human and mouse microarrays

(a) The co-DEGs in acute infection were identified in human and mouse microarrays. **(b)** The co-DEGs in persistent infection were identified in human and mouse microarrays.

as DDX58 (5.62-fold, Table S1), were identified in human arrays while some other unique classes of genes, including exocytosis-related genes such as RIMS3 (0.14 fold, Table S2), and immune genes such as CD68 (8.17-fold, Table S2), were identified in mouse arrays.

In the group of persistent infection, genes involved in organismal growth and development, such as COL1A1 (0.01-fold, Table S1), SERPINF1 (0.05-fold, Table S1) and COL1A2 (24.45-fold, Table S1), were mostly identified in human microarrays. Interestingly, COL1A1 and COL1A2 had the opposite fold change although they are two forms of collagen type I, and both are involved in skeletal system development. Some genes that may play important roles in the persistence of FMDV such as CHAC1 (5.15-fold, Table S1), FOS (5.64-fold, Table S1), CREB5 (0.10-fold, Table S2), ITSN1 (0.11-fold, Table S2) and RGS16 (6.54-fold, Table S2) were identified as well. The calcium-dependent cell-cell adhesion gene CDH11 (0.002-fold, Table S1), identified by using human microarray, was largely down-regulated in persistent infection. This might partially explain why persistently infected cells looked rounder and more scattered than acutely infected cells and normal BHK-21 cells (Huang *et al.*, 2011; Zhang *et al.*, 2013). The mitotic cytokinesis-related gene STAMBP (30.16-fold, Table S2), identified by using mouse microarray, was largely up-regulated in persistent infection. The up-regulation of this gene might contribute to the survival of the host cells after the lytic infection with FMDV.

Co-DEGs (common DEGs) were identified in human and mouse microarrays

Data analysis showed that 12 genes were commonly identified in human and mouse arrays, with 5 in the acute infection (Fig. 3a) and 7 in the persistent infection (Fig. 3b). In particular, there were seven genes that were found to be differentially expressed and had similar fold changes in both human and mouse arrays (Table 2). In acute infection, co-DEGs were related to DNA repair (Apex2), transcription (Mllt3) and signal transduction (Tle1). In persistent infection, co-DEGs were involved in organismal development (Sema4c), carbohydrate metabolic process (Ttn) and ubiquitin-dependent protein catabolic process (Usp11).

We then analyzed common GO biological processes (GO: BP) in human and mouse microarrays by using DAVID bioinformatics resources online tool (Huang da *et al.*, 2009a; Huang da *et al.*, 2009b). The 370 and 269 DEGs of human and mouse arrays in the acute infection group, as well as 506 and 483 DEGs in the persistent infection group were mapped to GOTERM_BP_FAT category. As a result, only up-regulated GO biological processes were found in either human or mouse arrays (Table 3). In acute infection, the bioactivity of host cells was clearly compromised. Gene functional clustering showed that cellular biosynthetic processes, macromolecule biosynthetic and metabolic processes were negatively regulated. Biological processes such as the regulation of protein localization, protein transport and post-Golgi vesicle-mediated transport, which may be used by viruses for their own replication cycle, including capsid packaging and virus particles release, were clearly strengthened. In persistent infection, gene functional clustering showed that biological processes such as heart development, muscle organ development, muscle tissue development and striated muscle tissue development were all involved in response to the viral persistent infection. These clustered GO biological processes might be a critical mechanism used by host cells to survive in the situation of viral persistence.

Gene expression validation by using real-time RT-PCR

To confirm the results obtained from microarray analysis, we performed qRT-PCR for a selected group of 33 DEGs.

Table 2. Seven DEGs were expressed in a similar trends in human and mouse arrays

Gene symbol	Gene description	GO biological process description	Fold changes	
			Human	Mouse
		Acute infection		
Apex2	apurinic/apyrimidinic endonuclease 2	DNA repair	2.27	5.59
Mllt3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3	DNA-dependent regulation of transcription,	0.39	0.27
Tle1	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	signal transduction	2.21	2.07
		Persistent infection		
Cep135	centrosomal protein 135kDa	–	0.38	0.40
Sema4c	sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4C	multicellular organismal development	0.24	0.28
Ttn	titin	carbohydrate metabolic process	3.41	2.20
Usp11	ubiquitin specific peptidase 11	ubiquitin-dependent protein catabolic process	0.50	0.32

Table 3. Common up-regulated GO biological processes were identified by human and mouse microarrays. The common GO biological processes were determined by DAVID bioinformatics resources. For analysis, the parameter "count" threshold is 2 and the "EASE" is 0.1

Biological process	Up-regulated DEGs in human microarray	Up-regulated DEGs in mouse microarray
	Acute infection	
Establishment of protein localization	SNX19, GNPTG, MCM3AP, TMCO6, SEC24A, ATG9A, C16ORF70, IPO11, TIMM50, HLA-DMA, PEX7, SCFD1, BACE2, NPM1, ARCN1, MGEA5, NCKIPSD, RAMP1, COPE, HDAC6	RAB4B, ARF5, SNX3, VPS37D, ERC1, MON2, COG1, SNX11
Negative regulation of biosynthetic process	SBNO2, ZNF593, PDGFB, DEDD, TLE1, MBD3, MXD4, RPS14, BACE2, MGEA5, COMMD7, HDAC8, DNAJB6	GMNN, TH1L, HES7, TLE1, NFIC, ADIPOQ, FOXP2, CDT1
Negative regulation of cellular biosynthetic process	SBNO2, ZNF593, PDGFB, DEDD, TLE1, MBD3, MXD4, RPS14, BACE2, MGEA5, COMMD7, HDAC8, DNAJB6	GMNN, TH1L, HES7, TLE1, NFIC, ADIPOQ, FOXP2, CDT1
Negative regulation of macromolecule biosynthetic process	SBNO2, ZNF593, DEDD, BACE2, RPS14, MGEA5, TLE1, MBD3, COMMD7, HDAC8, DNAJB6, MXD4	GMNN, TH1L, HES7, TLE1, NFIC, FOXP2, CDT1
Negative regulation of macromolecule metabolic process	CLN3, SBNO2, ZNF593, DEDD, TLE1, ANAPC10, MBD3, MXD4, BACE2, RPS14, NPM1, MGEA5, COMMD7, HDAC8, RPS27A, BUB3, DNAJB6, HDAC6	HNF4A, GMNN, TH1L, HES7, TLE1, NFIC, FOXP2, CDT1
Post-Golgi vesicle-mediated transport	SCFD1, GBF1, C16ORF70, MUTED	CHIC2, MON2
Protein transport	SNX19, GNPTG, MCM3AP, TMCO6, SEC24A, ATG9A, C16ORF70, IPO11, TIMM50, HLA-DMA, PEX7, SCFD1, BACE2, NPM1, ARCN1, MGEA5, NCKIPSD, RAMP1, COPE, HDAC6	RAB4B, ARF5, SNX3, VPS37D, ERC1, MON2, COG1, SNX11
	Persistent infection	
Heart development	CHD7, ID1, PPP3CB, ID3, TTN, FOXP1	MYO18B, NOTCH1, NODAL, TTN, POFUT1, GJA5
Muscle organ development	IGSF8, CRYAB, FHL1, MET, TEAD4, PPP3CB, MYLPF, HBEGF, TTN, CDK5, FOXP1	LIF, MYO18B, MUSK, TTN, CHAT, FOXP2
Muscle tissue development	IGSF8, PPP3CB, MYLPF, TTN, CDK5, FOXP1	MYO18B, MUSK, TTN, CHAT, FOXP2
One-carbon metabolic process	ATF7IP, FOS, GSPT1, AHCYL1, PCMT1	SUV420H2, TRMT11, AHCYL2, HELLS
Striated muscle tissue development	IGSF8, PPP3CB, MYLPF, TTN, CDK5, FOXP1	MYO18B, MUSK, TTN, CHAT, FOXP2

Three of them (Apex3, Ttn and Usp11) were selected from Table 2 (Co-DEGs). Fifteen genes were selected from Table S1 (Significant DEGs in human microarray) and the remaining ones were from Table S2 (Significant DEGs in mouse microarray). The data showed that all genes selected had similar expression patterns in both human and mouse microarrays (Fig. 4). Especially, the expression patterns of 7 genes (HPS1, DDX58 and KPTN in acute infection (Fig. 4a));

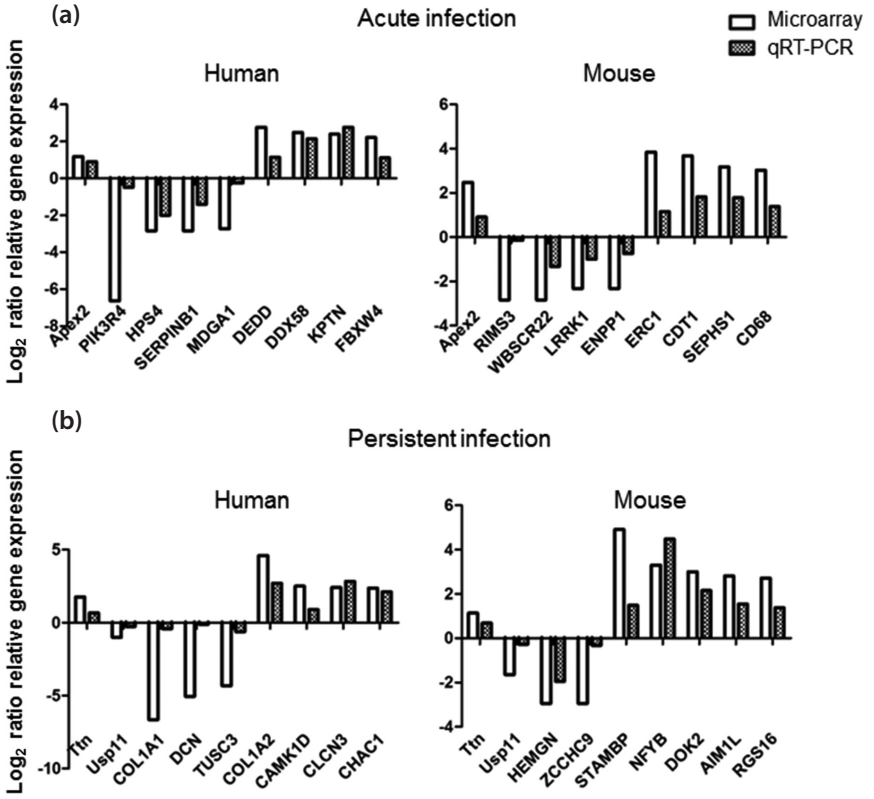

Fig. 4

Validation of gene expression in human and mouse microarrays

(a) Validation of gene expression in acute infection by using qRT-PCR. **(b)** Validation of gene expression in persistent infection by using qRT-PCR. Mock-infected sample was used a control for the FMDV infected samples. The transcript levels revealed by qRT-PCR were normalized against the housekeeping gene GAPDH. The y-axis represented $\log_2$ ratio of fold changes. Each reaction was repeated in duplicates.

CLCN3, CHAC1, NFYB, and DOK2 in persistent infection (Fig. 4b) were almost consistent with those in microarrays. Although the fold change of most of genes in qRT-PCR test was a little smaller than that in microarray, the expression patterns of these genes were quite similar.

Discussion

Due to the lack of native gene arrays for Syrian hamster, we used human and mouse microarrays to study the gene expression profiles in BHK-21 cells that were acutely and persistently infected with FMDV. On average, about 10% of the total probes in each array could be obviously detected in virus-infected or uninfected BHK-21 cell samples. Furthermore, expression changes of some genes in the FMDV-infected cells, compared with the uninfected BHK-21 cells, could be detected by using either human or mouse microarrays or the combination of the two types of microarrays. This suggested that the corresponding sequences of Syrian hamster at the specific locations are conserved with those from either human or mouse.

We used two-channel microarrays to hybridize the cDNAs from virus-infected and uninfected cells. The cDNAs of uninfected BHK-21 cell samples were labeled with Cy3 in red, while the cDNAs of virus-infected ones were labeled with Cy5 in green. Therefore, microarrays with probes hybridizing cDNAs of both virus-infected and uninfected samples would show yellow color. MA Plot not only showed the results of hybridization from two-channel microarrays, but also presented the fluorescence intensity of Cy3 and Cy5 channels. Hence, this could be an appropriate method to provide us an overall view of the cross-species hybridization results.

As we have shown, in both acute and persistent infections human microarrays hybridized many more (about 3 times) targets compared to mouse microarrays. In order to further investigate whether a transcriptome of Syrian hamster had higher homology to that of human, we cloned ten genes from BHK-21 cells, sequenced them and ran sequence alignment among Syrian hamster, human and mouse. The result

showed that nine of the ten cloned genes had higher identity with mouse as compared to human corresponding genes (Table S3). These data demonstrated that Syrian hamster was more homologous to mouse than human, although human microarrays could hybridize more cDNAs of BHK-21 cells than mouse microarrays.

It has been recognized that genes with striking fold changes in microarray test are usually more valuable to investigators. We defined significant differentially expressed genes, of which the fold changes were larger than 4 or smaller than 0.25. Common and unique significant genes that might be related to the mechanisms underlying their own specific infection patterns were identified in both human and mouse microarrays. For example, in acute infection, ubiquitin-related genes, such as RNF139 and KPTN, and virus-responsive genes, such as DDX58, were identified in human microarray, while exocytosis genes, such as RIMS3, and immune genes such as CD68, were identified in mouse microarray. In persistent infection, multicellular organismal development genes, such as COL1A1, SERPINF1 and COL1A2, and calcium-dependent cell-cell adhesion genes, such as CDH11 were identified in human microarray. The most significant up-regulated gene STAMBP, which may participate in assisting persistently infected cells to survive, was identified in mouse microarray.

We then analyzed the common DEGs (co-DEGs) shared by human and mouse microarrays. Seven genes found to be differentially expressed in persistent infection had similar fold changes in human and mouse arrays, with 3 of them in acute infection and 4 in persistent infection. These co-DEGs could be valuable in further investigation. To further confirm the results obtained with the microarray analysis, we performed qRT-PCR for a selected group of DEGs. We selected 33 genes, 3 (Apex2, Ttn and Usp11) of which were selected from Table 2 and the remaining were selected from Table S1 and Table S2. The results of qRT-PCR for acute and persistent infections presented great consistency in both human and mouse microarrays (Fig. 4, Table S5).

Finally, we used DAVID bioinformatics resources tool to analyze the common genes involved in biological processes between human and mouse microarrays. The gene functional clustering analysis of these two microarrays gave us better understanding of the unique biological processes taken by host cells in acute and persistent infections. Evidently, various biosynthetic and metabolic processes were negatively regulated in acute infection. Genes involved in multicellular organismal development processes were clustered in persistent infection. These results are valuable to explore the possible mechanisms taken by host BHK-21 cells in each infection pattern.

In summary, microarray studies in four BHK-21 cell samples (infected or uninfected) were performed with human and mouse oligonucleotide arrays. Surprisingly, in hybridization with the cDNAs from FMDV-infected BHK-21 cells, human arrays are more informative than mouse arrays. Similar hybridization results were also reported in a previous study. The sequence alignments of ten genes cloned from BHK-21 cells confirmed that Syrian hamster is evolutionarily closer to mouse than human beings. Therefore, the combined use of human and mouse microarrays is more informative than the use of either array, which would be helpful for investigators to establish more complete gene expression profiles for other species.

Acknowledgement. The authors gratefully acknowledge KangChen Bio-tech (Shanghai, China) and China Infrastructure of Cell Line Resources for technical supports. This work was supported by the National Natural Sciences Foundation of China (No. 31370185) and the National Basic Research Program of China (No. NSTI-CR14, NSTI-CR15).

Supplementary information is available in the online version of the paper.

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Supplementary information

Cross-species hybridization of foot-and-mouth disease virus-infected BHK-21 cells using human and mouse oligonucleotide microarrays

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Received August 8, 2016; revised December 19, 2016; accepted June 27, 2017

Table S1. Significant DEGs in human microarray

Fold change	Gene description	Gene symbol	GO biological process description	UniGene ID
Acute infection				
0.01	phosphoinositide-3-kinase, regulatory subunit 4	PIK3R4	protein amino acid phosphorylation	Hs.149032
0.14	Hermansky-Pudlak syndrome 4	HPS4	protein targeting	Hs.474436
0.14	Serpin B1	SERPINB1	–	–
0.15	MAM domain containing glycosylphosphatidylinositol anchor 1	MDGA1	–	Hs.437993
0.15	chromosome 12 open reading frame 36	C12orf36	–	Hs.448717
0.18	solute carrier family 2, member 8	SLC2A8	carbohydrate metabolic process	Hs.179522
0.18	solute carrier family 38, member 4	SLC38A4	ion transport	Hs.446077
0.18	ring finger protein 139	RNF139	ubiquitin cycle	Hs.632057
0.19	SET binding protein 1	SETBP1	–	Hs.435458
4.17	transmembrane 6 superfamily member 1	TM6SF1	–	Hs.513094
4.44	ribosomal protein L37	RPL37	translation	Hs.80545
4.63	F-box and WD repeat domain containing 4	FBXW4	ubiquitin cycle	Hs.500822
4.66	coiled-coil domain containing 106	CCDC106	–	Hs.82482
4.89	transmembrane and coiled-coil domains 6	TMCO6	–	Hs.651145
5.28	kaptin	KPTN	cell motility	Hs.25441
5.62	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	DDX58	response to virus	Hs.190622
5.90	zinc finger, DHHC-type containing 5	ZDHHC5	–	Hs.27239
6.77	death effector domain containing	DEDD	transcription	Hs.517342
Persistent infection				
0.002	cadherin 11	CDH11	cell adhesion	Hs.116471
0.01	collagen, type I, alpha 1	COL1A1	skeletal system development	Hs.172928
0.03	defective in cullin neddylation 1	DCN	carbohydrate metabolic process	Hs.503716
0.03	metallothionein 1E	MT1E	negative regulation of growth	Hs.534330
0.04	islet cell autoantigen 1	ICA1	neurotransmitter transport	Hs.487561
0.05	oxysterol binding protein-like 6	OSBPL6	lipid transport	Hs.318775
0.05	tumor suppressor candidate 3	TUSC3	post-translational protein modification	Hs.426324
0.05	SAC3 domain containing 1	SAC3D1	negative regulation of JAK-STAT cascade	Hs.23642

Table S1 (continued)

Fold change	Gene description	Gene symbol	GO biological process description	UniGene ID
0.05	serpin peptidase inhibitor, clade F	SERPINF1	multicellular organismal development	Hs.532768
5.15	ChaC, cation transport regulator homolog 1	CHAC1	negative regulation of Notch signaling pathway	Hs.155569
5.37	chloride channel 3	CLCN3	ion transmembrane transport	Hs.481186
5.62	solute carrier family 24, member 3	SLC24A3	transmembrane transport	Hs.654790
5.64	upstream transcription factor 2	FOS	toll-like receptor signaling pathway	Hs.454534
5.67	plasma glutamate carboxypeptidase	PGCP	proteolysis	Hs.156178
5.69	calcium/calmodulin-dependent protein kinase ID	CAMK1D	protein phosphorylation	Hs.659517
6.56	Ras-related associated with diabetes	RRAD	GTP catabolic process	Hs.1027
6.80	adenosylhomocysteinase-like 1	AHCYL1	mRNA polyadenylation	Hs.485365
24.45	collagen, type I, alpha 2	COL1A2	skeletal system development	Hs.489142

Table S2. Significant DEGs in mouse microarray

Fold change	Gene description	Gene symbol	GO biological process description	UniGene ID
Acute infection				
0.12	adult male cecum cDNA, unclassifiable	9130221J18RIK	–	Mm.207250
0.14	regulating synaptic membrane exocytosis 3	RIMS3	exocytosis	Mm.479706
0.14	Williams Beuren syndrome chromosome region 22	WBSCR22	rRNA processing	Mm.439878
0.20	leucine-rich repeat kinase 1	LRRK1	protein phosphorylation	Mm.33284
0.20	ectonucleotide pyrophosphatase/phosphodiesterase 1	ENPP1	generation of precursor metabolites and energy	Mm.485841
0.24	hypothetical LOC432488	LOC432488	–	Mm.32833
0.24	7 days embryo whole body cDNA, unclassifiable	C430019N01RIK	–	Mm.447782
0.24	TGF-beta activated kinase 1/MAP3K7 binding protein 2	TAB2	MyD88-dependent toll-like receptor signaling pathway	
0.25	absent in melanoma 1-like	AIM1L	–	Mm.240080
8.17	CD68 antigen	CD68	cellular response to organic substance	Mm.15819
9.07	selenophosphate synthetase 1	SEPHS1	cellular protein modification process	Mm.34329
10.37	T-cell leukemia/lymphoma 1B, 1	TCL1B1	–	Mm.241933
11.43	adult male testis cDNA, unclassifiable	1700018G05RIK	–	Mm.138009
11.67	ADAM Metallopeptidase Domain 28	ADAM28	proteolysis	Mm.117450
12.81	chromatin licensing and DNA replication factor 1	CDT1	DNA replication checkpoint	Mm.21873
13.73	retinol binding protein 7, cellular	RBP7	–	Mm.46023
14.42	ELKS/RAB6-interacting/CAST family member 1	ERC1	regulation of transcription, DNA-templated	Mm.288860
16.46	ADAMTS-like 1	ADAMTSL1	–	Mm.331690
Persistent infection				
0.10	cAMP responsive element binding protein 5	CREB5	positive regulation of transcription, DNA-templated	Mm.321138
0.11	mus musculus expressed sequence AW555464	AW555464	–	Mm.23689
0.11	intersectin 1 (SH3 domain protein)	ITSN1	positive regulation of apoptotic process	Mm.40546
0.12	transmembrane protein 62	TMEM62	–	Mm.315212

Table S2 (continued)

Fold change	Gene description	Gene symbol	GO biological process description	UniGene ID
0.13	Eph receptor B3	EPHB3	transmembrane receptor protein tyrosine kinase signaling pathway	Mm.6972
0.13	hemogen	HEMGN	multicellular organismal developmen	Mm.25793
0.13	eyes absent 1 homolog	EYA1	establishment of mitotic spindle orientation	Mm.250185
0.13	N-sulfoglucosamine sulfohydrolase	SGSH	glycosaminoglycan catabolic process	Mm.103788
0.13	zinc finger, CCHC domain containing 9	ZCCHC9	negative regulation of phosphatase activity	Mm.52356
6.45	mus musculus RIKEN cDNA E130309D02 gene	E130309D02RIK	–	Mm.440105
6.54	regulator of G-protein signaling 16	RGS16	positive regulation of GTPase activity	Mm.181709
6.69	junction plakoglobin	JUP	negative regulation of transcription from RNA polymerase II promoter	Mm.299774
6.95	sugano mouse kidney mkia Mus musculus cDNA clone	AI987986	–	Mm.443881
7.01	absent in melanoma 1-like	AIM1L	–	Mm.240080
7.65	gamma-aminobutyric acid (GABA) A receptor, pi	GABRP	transport	Mm.99989
8.03	docking protein 2	DOK2	signal transduction	Mm.243323
9.85	nuclear transcription factor-Y beta	NFYB	transcription, DNA-templated	Mm.245998
30.16	STAM binding protein	STAMBP	mitotic cytokinesis	Mm.32801

Table S3. Homology comparation of Syrian hamster genes to human and mouse by sequence alignment

	Hamster cloned gene	Cloned sequence lenth (bp)	Species	Genebank number	Gene lenth (bp)	Sequence identity (%)	More close to hamster
1	EBP	693	Human	NM_006579	1191	80.09	Mouse
			Mouse	NM_007898	1755	87.30	
2	USP11	1661	Human	NM_004651	3300	84.23	Mouse
			Mouse	NM_145628	3464	92.66	
3	GNG12	219	Human	NM_018841	4427	86.30	Mouse
			Mouse	NM_001177560	4264	94.98	
4	FKBP1A	327	Human	NM_000801	1643	94.80	Mouse
			Mouse	NM_008019	1657	96.02	
5	TUBB2A	1317	Human	NM_001069	1621	90.66	Mouse
			Mouse	NM_009450	1638	92.94	
6	TUBA1B	1349	Human	NM_006082	1771	94.96	Mouse
			Mouse	NM_011654	1747	96.44	
7	APEX2	98	Human	NM_014481	2095	92.86	Human
			Mouse	NM_029943	1903	91.84	
8	LEPREL4	69	Human	NM_006455	2619	91.30	Mouse
			Mouse	NM_176830	1903	92.75	
9	RPL34	118	Human	NM_000995	918	94.92	Mouse
			Mouse	NM_026724	628	96.61	
10	RPS23	189	Human	NM_001025	3325	89.42	Mouse
			Mouse	NM_024175	572	94.18	

EBP1:
ATGACCACCAATTCGGGCCCGGTACACCCAT
ACTGGCCCAGGCACCTGAAGCTGGACAACTTT
GTGCCTAATGACCTCCCTACCTGGAATATCCT
GGTTGGCCTATTCTCCATCTCTGGATTCCTAATTG
TGGTCATGTGGCTGTTGTCCAGTCGAGTTTCCGT
TGCCCCACTTGGAATTTGGCGTCGACTGGCCCTGT
GCTGGTTTGCTGTGTGTGCGTTCATTCACCTTGT
GATCGAGGGTTGGTTCTCTGTATACCATGACATC
CTTCTTGAAGACCAAGCCGTCTTATCCCAACTCT
GGAAAGAGTATTCCAAGGGAGACAGCCGA
TATATACTTAATGACGGCTTCATCGTCTCTAT
GGAGACTGTCACAGCTGTTCTCTGGGGAC
CACTCAGCCTGTGGGTAGTGATTGCCTTTCTC
CGCCAACAACCTTACCGCTTTATCCTACAGCTCGT
GATCTCTGTGGGCCAGATATACGGGGATGTGCTG
TACTTCCTGACTGAGCTTCGTGATGGATTCCAGCAT
GGGGAGCTCGGCCACCCCCTTTATTTCTGGTTT
TACTTTGTTATCTTGAATGGCATATGGCTGGTGG
TACCTGCAATCCTCGTGCTTGATGCTATAAAG
CATCTCGCTCACGCCCAGAGCATGTTGGACAG
CAAGGTTATGAAAACTAAGAGCAAACATAACTAA

USP11:
ATGGCGGCGGTCGCAGCGGACCCAGCTGCAGC
TGCCATCCCGGCCTCGGCCGAGGAGGAAGAG
AGACAGCACGAGGCAATGCCAGACCTGGACGA
GCAGTGGCGCCAAGTCGAGAATGGGCG
AGAGCGTCCACTGCGAGCTGGCGAAAGCTGG
TTCCTTGTGGAGCAACACTGGTTTAAACAG
TGGGAGGTGTACGTGAAGGGAGGGGACCAGGA
TGCTAACACCTTTCCTGGCTGTATCAACAATGC
TGGGCTCTTCGAAGATCAGATAAACTGGCACC
TCAGGGAGGGACTGCTAGAAGGAAATGATTATG
TGCTGCTCCCAGCGACTTCATGGAATTACTTGG
TCAGCTGGTATGGCCTAGAAGATGGCCAGCCA
CCTATTGAGCGCAAGGTCATAGAACTTTCTGG
TGTCCCAAAGGTGGAATTATACCCAATAGAGCTGC
TGCTTGTCCAGCACAGTGATATGGAAACAGCTC
TCACCATTCAGTTCAGCCACACCGATTCTC
TGGACGTAGTCTTGCAAACAGCTCGGGAGCAGT
TTCTGGTGGAGCCTCAGGAAGACACACGCCTC
TGGATCAAGAACTCCGAGGGCTCCTTGGATCGAT
TGTGTAACACACAAATCACACTGCTCGATGCCT
CCCTTGAGACTGGACAGTTGGTCATCATGGAAA
CCCGAAACAAAGATGGCACTTGGCCCAGCGC
TCAGCTGTGTGGCATGGACAACATGCCAGAAGA
GGATGAAGACTTCCAGGGCCAGCCGGGCATC
TGTGGCCTTACCAATCTGGGCAACACGTGC
TTCATGAACTCGGCCCTACAGTGCCTCAGCAA
TGTGCCACAGCTCACGGAGTACTTTCTCAACAA
CCGCTACCTGGAGGAGCTCAACTTCTGGAACCCT
TTAGGCATGAAGGGTGAGCTTGCTGAGGCCTA
TGCAGATCTGGTAAAGCAGACCTGGTCTGGCTA
CCACCGCTCCATTGTGCCGAATGTGTTCA
AGAACAAGGTTGGCCATTTTGCATCCCAGTTTC
TGGGGTACCAGCAACATGACTCACAGGAACTGT
TGTCATTCCTTCTGGATGGGCTACATGAGGACC
TCAATCGTGTCAAAAAGAAAGAATATGTTGAGC
TGTGCGATGGTGCTGGGCGCCCGGATTTGGAAG
TGGCTCAGGAAGCCTGGCAAAACCACAAACGA
CGAAATGATTCTGTGATTGTGGATACTTTCCA
TGGCCTCTTCAAATCTACTCTGGTGTGCCCTGAT
TGTGGCAATGTATCTGTGACCTTTGACCCCTTC
TGCTACCTCAGCGTCCCACTGCCTGTCTGCT
CCAGGAGGGTCTTGGAGGTCTTCTTTGTCCCCA
TGGATCCCCGCCGCAAGCCAGAACAGCACCGGGT
TGTGGTCCCCAAGAAAGGCAATATTTCAGATCTG
TGTGTGGCTCTGTCCACACACACAAGTGTTGCA
CCAGACAAGATGATAGTAGCTGATGTCTTCAGTCAC
CGATTCTATAAGCTCTACCAGCTGGAGGATCCTCT
GAGTAGCATCTTGGATCGGGATGATATCTTTGTATAT
GAGGTGACTGGTCGGATTGAGCCTGTTGAGGGT
TCAAGAGATGATATCGTGGTTCCTGTTTACCTGCGA
GAGCGCACCCCATCCCGAGACTTCGACAACTC

GNG12:
ATGTCCAGCAAGACGGCAAGCACCAACAACA
TAGCCCAGGCCAGGAGAACGGTGCAGCAGTTG
AGACTGGAAGCCTCCATCGAAAGAATAAAGGTCT
CCAAAGCATCTGCAGACCTGATGTCATACTGTGA
GGAGCATGCCCGGAGCGACCCTCTACTGA
TGGGCATACCGACCTCGGAAAACCCATTCAAGG
ATAAGAAGACCTGCATCATCTTATAG

FKBP1A:
ATGGGAGTGCAGGTGGAAACCATCTCTCCTGG
AGACGGACGCACCTTCCCGAAGCGCGGCCAGA
CCTGCGTGGTGCACTACACGGGAATGCTTGAA
GATGGAAAGAAATTTGATTCTTCTCGGGACA
GAAACAAGCCTTTTAAGTTTATGCTAGGCAAG
CAGGAGGTGATCCGAGGCTGGGAAGAAGGGG
TAGCCCAGATGAGTGTGGGTCAGAGAGCCAAACT
GACAATCTCCCCAGATTATGCCTATGGAGCCACT
GGGCACCCAGGCATCATCCCACCACATGCCACTCT
GGTTTTTGATGTGGAGCTTCTAAAACTGGAATGA

TUBB2A(beta-tubulin)
ATGCGTGAGATCGTGCACATCCAGGCGGGCCAG
TGCGGCAACCAGATCGGCGCTAAGTTTTGGGA
GGTGATAAGCGATGAGCATGGCATCGACCCCA
CCGGCAGTTACCATGGTGACAGTGATTTGCAGC
TGGAGAGAATCAATGTGTACTACAATGAAGC
TGCTGGCAACAAATATGTACCTCGGGCCAT
CCTGGTGGACCTGGAGCCTGGCACCATGGAC
TCAGTGAGGTCAGGACCCTTCGGCCAGATC

TTCAGGCCAGACAACTTTGTTTTCGGCCAGAGCG
GTGCAGGAAACAACTGGGCAAGGGCCACTACACA
GAGGGCGCTGAGCTGGTGGACTCCGTCCTGGATG
TAGTGAGGAAGGAGTCTGAAAGCTGTGACTGTCTC
CAGGGCTTCCAGCTGACCCACTCACTGGGGGGAG
GCACTGGCTCAGGCATGGGGACCCTGCTCAT
CAGCAAGATCCGAGAGGAGTACCCAGACCGCAT
CATGAACACCTTCAGTGTCATGCCCTCACCCAAG
GTCTCTGACACTGTGGTGGAGCCCTATAATGCCAC
CCTCTCCGTGCACCAGCTGGTGGAGAACACAGAT
GAAACCTATTCCATTGACAACGAGGCCCTGTACGA
CATCTGCTTCCGCACCCTCAAGCTGACCACACCCA
CATATGGCGACCTCAACCACCTGGTGTCAGCCAC
CATGAGTGGGGTGACCACCTGCCTGCGCTTC
CCAGGCCAGCTGAACGCAGACCTGCGCAAGCT
GGCCGTGAACATGGTGCCCTTCCCACGCCTGCACT
TCTTCATGCCAGGCTTCGCGCCTCTGACCAGCAG
GGGCAGCCAGCAGTACCGAGCCCTGACAGTGCCT
GAGCTCACCCAGCAGATGTTCGATGCCAAGAACAT
GATGGCCGCCTGTGATCCCCGCCATGGGCGCTACTT
GACAGTGGCTGCTGTGTTCCGGGGCCGCATGTC
TATGAAGGAGGTGGACGAACAGATGCTTAATGTC
CAAAACAAGAACAGCAGCTACTTTGTTGAGTGGAT
TCCCAACAATGTGAAAACAGCTGTCTGTGACAT
TCCACCTCGGGGTCTAAAAATGTCTGCCACCT
TCATCGGCAACAGCACCGCCATTCAGGAGCTGT
TCAAACGCATCTCTGAGCAGTTCACAGCCATGT
TCCGACGCAAGGCTTTCCTGCACTGGTACACG
GGTGAGGGCATGGATGAGATGGAGTTCACTGA
GGCTGAGAGCAACATGAATGACCTGGTGTCTGAG
TACCAGCAGTACCAGGATGCCACGGCTGAGGAG
GAGGGCGAGGACGAGGCGTAGG

TUBA1B(alpha-tubulin)
ATGCGTGAGTGCATCTCCATCCACGTTGGCCA
GGCTGGTGTCCAGATCGGCAATGCCTGC
TGGGAGCTCTACTGCCTGGAACATGGCAT
CCAGCCTGACGGCCAGATGCCAAGTGACAAGA
CCATTGGGGGAGGAGATGACTCCTTCAACA
CCTTCTTCAGTGAGACAGGCGCTGGCAAGCA
TGTGCCCCGGGCTGTGTTCGTAGACCTGGAA
CCCACAGTTATCGATGAAGTTCGCACTGGTACCTA
CCGCCAGCTCTTCCACCCTGAGCAGCTCATCACA
GGCAAGGAAGATGCTGCCAACAACTATGCCCG
TGGCCACTACACCATTGGCAAGGAGATCAT
TGATCTTGTCTTGGACAGAATTCGCAAACTGGC
TGACCAGTGCACGGGTCTTCAGGGCTTCCTGGTT
TTCCACAGCTTTGGTGGGGGAACTGGCTCTGGG
TTCACCTCCCTGCTGATGGAGCGGCTCTCTGT
CGATTATGGAAAGAAGTCCAAGCTGGAGTTCT
CCATCTACCCAGCCCCCCAGGTTTCCACCGC
TGTGGTTGAGCCCTACAACTCCATCCTCACCA
CCCACACCACCCTGGAGCACTCTGATTGTGCC
TTCATGGTAGACAATGAGGCCATCTATGACATC
TGTCGTAGAAACCTCGACATTGAGCGCCCCA
CCTACACTAACCTTAACCGCCTCATTAGCCAGAT
TGTGTCTTCCATCACTGCTTCCCTCAGATTTGA
TGGGGCCCTGAATGTTGACCTGACAGAGTTCC
AGACCAACCTGGTACCCTACCCTCGCATCCAT
TTCCCTCTGGCCACATATGCCCCTGTCATCTCTGC
TGAGAAAGCCTACCATGAGCAGCTTTCTGTAGC
AGAGATCACCAATGCCTGCTTTGAGCCAGCCAA
CCAGATGGTGAAATGTGACCCTCGCCATGGTAAA
TACATGGCTTGCTGCCTGCTGTACCGTGGGGATG
TGGTTCCCAAAGATGTCAATGCTGCCATTGCCA
CCATCAAGACCAAGCGCAGCATCCAGTTTG
TGGACTGGTGCCCCACTGGCTTCAAGGTTGGCA
TTAACTACCAGCCTCCCACCGTGGTACCTGG
TGGAGACCTGGCCAAGGTCCAGAGAGCTGTG
TGCATGCTGAGCAATACCACAGCCATCGCTGA
GGCCTGGGCTCGCCTAGATCACAAGTTTGATC
TGATGTATGCCAAGCGTGCCTTTGTGCACTGGTA
TGTGGGTGAGGGCATGGAGGAAGGGGAGTTCTC
TGAGGCCCGTGAAGACATGGCTGCCCTAGAGAA
GGATTATGAGGAGGTTGGTGTGGATTCTGTTGA
GGGAGAGGGTGAGGAAGAAGGAGAAGA

APEX2
ATTCGTCTGTCTCCAGGAAACCAAAGTGACCAG
AGATGCTCTGACGGAGCCCCTGGCTATTGTTGA
GGGCTATAACTCCTATTTCAGCTTCAGCCGCA

EBP
ACTGTCCCTGTGCTGGTTTGCTGTGTGTGCG
TTCATTCACCTTGTGATCGAGGGTTGGTTCTCTG
TGTACCATGACATCCTTCTTGAAGACTAAGCCGTC
TTATCCCAACTCTGGAAAGAGTATTCCAAGGG
AGACAGCCGATACATC

LEPREL4
GGTGGCTACTTCGTGGACAAGTTTGTGGCCACCA
TGTATCACTACCTGCAGTTTGCCTACTACAAATGA

RPL34
CAGCGTTTGACATACCGACGTAGGCTTTCCT
ACAACACAGCCTCTAACAAAACTAGGCTGTCT
CGAACCCCTGGCAACAGGATCGTTTACCTTTATA
CCAAGAAGGTTGGGAAAGCAC

RPS23
GATGGGCAAGTGTCGCGGTCTCCGTACTGCCC
GGAAGCTCCGCAGTCACCGACGGGACCAGAAG
TGGCATGATAAGCAGTACAAGAAAGCCCACC
TGGGCACAGCCCTGAAGGCCAACCCCTTCGGAGG
TGCCTCTCATGCGAAGGGAATTGTGCTGGAAAAA
GTAGGGGTTGAAGCCAAACAGCCAAA

Table S4. Primers for real-time RT-PCR validation

Gene	Forward primer	Reverse primer	Product lenth (bp)
Acute infection			
Apex2	CGTCTGTCTCCAGGAAACCA	GCGGCTGAAGCTGAAATAGG	94
Human microarray			
PIK3R4	AAAATAAGGTTCTGGGACT	GTAGGACACGGCTGGA	81
HPS4	GTGCGTGAACTGGAAACTC	GGTGGCTTCGGATAGGT	91
SERPINB1	TGCCAGGACGACACTC	TGGTGAAGGCTGCTGTA	110
MDGA1	GTGGCATCCCAGATAAGGC	CCAGAGTTTCGTTCACAGACAG	87
DEDD	GTTTCCTGTTGCCACTTAC	CCTTACAAAGGCTATGCTC	102
DDX58	CTGGTGGCTTGTTGCT	GATTCTGCCGCTGTTC	130
KPTN	GGTGGTGGGCATCACAT	CCAGGCTCATAGTCACAGTAAA	81
FBXW4	TCCTCCTACTATGGTGTTGTG	AGATGCCTGGTGGTGAAT	125
Mouse microarray			
RIMS3	AGCAGCATTTCTGTCGTG	AGAGGCGGCGTATTCA	134
WBSCR22	GAAGAAGAGCAGGGAATGG	CCGTGTACTGGGTGTCAGG	86
LRRK1	AGGGCACAGAAGACAAACA	TCCCAAACCGCCAGTA	81
ENPP1	TCCTCGCTCCACCAA	ACATAGGCACGATATTAGA	81
ERC1	ATGAGTGACCGAATACAGC	TCAAGATTTCCAAGAGGC	100
CDT1	GGCTTTCCTTGCTTCTTT	AGGCACCTCGTCCACAT	91
SEPHS1	TAAACTCGCCCATCCT	ACGCTTCAGCCTCTTC	90
CD68	ATTACCCGAGGACGACA	TCAGTTGCGAGTTGAAGA	102
Persistent infection			
Titin	TGCCTAAGACAGAACACG	GCATGAATTTCGATACCA	160
Usp11	CTTTCTCAACAACCGCTAC	ACATTCGGCACAATGGA	135
Human microarray			
Col1a1	ATACCTGCTTGATCTGTATTTGC	TCCCTCGACTCCTATGAGTTC	167
Chac1	TTGCCTGCCGAGGTTTC	GCTTCCAGGTGCTCATCC	103
Col1a2	CCTAGCAACATGCCAATC	GCAAAGTTCCCAATAAG	216
DCN	ACTCTGAAGAAGGGCAACG	TTCCCACTCCACAAAGGTAT	257
TUSC3	GCGTTACTCATCTGCTTTC	CTCTTGGGTCTGCCTTT	238
CLCN3	TGCTATGCCTCTGCG	GTGTCACCGTCTAACAAAT	190
CAMK1D	CGAGGAGTCCAAGATAAT	TTCAGGAGCGACATAGC	106
Mouse microarray			
RGS16	ACAGTTGGCTTTGGTCATAC	AATCATATTGGGCTCGTTG	177
Stambp	CGTTACTTTCGTTCTGGTG	TTCTTCGGCTTTAGGGA	213
HEMGN	ATGGACCTAAAGACTGCC	AAATTCTGAGGAATCCCTAC	128
ZCCHC9	CAACAGACAGCCAGGAA	ACAGCGGTAACAAATGC	203
AIM1L	ATCCTCCGAACTCACCG	CCTCGTCCTCTTCTGATGC	148
DOK2	CCTCCTACGACTTTCCCACT	CACGAGCCAAGGAACCA	112
NFYB	ATTACGACCACTCTGCC	CTCCCTTTCCTATGTGC	126
House keeping			
GAPDH	AAGGCCATCACCATCTTCCA	GCCAGTAGACTCCACAACATAC	87

Table S5. Gene expression validation for human and mouse microarrays

Gene symbol	Microarray	RT-PCR
Acute infection		
Human microarray		
Apex2	2.27	1.88±0.26
PIK3R4	0.01	0.71±0.02
HPS4	0.14	0.25±0.03
SERPINB1	0.14	0.38±0.01
MDGA1	0.15	0.84±0.08
DEDD	6.77	2.21±0.13
DDX58	5.62	4.41±0.50
KPTN	5.28	6.76±0.10
FBXW4	4.63	2.17±0.09
Mouse microarray		
Apex2	5.59	1.88±0.26
RIMS3	0.14	0.91±0.02
WBSCR22	0.14	0.40±0.04
LRRK1	0.20	0.50±0.03
ENPP1	0.20	0.60±0.10
ERC1	14.42	2.22±0.06
CDT1	12.81	3.52±0.15
SEPHS1	9.07	3.43±0.17
CD68	8.17	2.62±0.05
Persistent infecion		
Human microarray		
Ttn	3.41	1.61±0.19
Usp11	0.50	0.83±0.02
COL1A1	0.01	0.75±0.17
DCN	0.03	0.93±0.04
TUSC3	0.05	0.65±0.02
COL1A2	24.45	6.54±0.60
CAMK1D	5.69	1.88±0.17
CLCN3	5.37	7.09±3.48
CHAC1	5.15	4.34±0.47
Mouse microarray		
Ttn	2.20	1.61±0.19
Usp11	0.32	0.83±0.02
HEMGN	0.13	0.26±0.04
ZCCHC9	0.13	0.80±0.09
STAMBP	30.16	2.80±0.34
NFYB	9.85	22.27±2.95
DOK2	8.03	4.46±0.87
AIM1L	7.01	2.92±0.42
RGS16	6.54	2.60±0.04

cDNA of was prepared from three independent BHK-21 cultures and used in qRT-PCR experiments using the SYBR green method and specific primers for homologous sequences of human and mouse. qRT-PCR levels of RNA for a given gene were normalized against the housekeeping gene GAPDH, and levels in FMDV infected samples expressed relative to the expression level in the corresponding Mock infected samples. Mean relative expression levels of triplication experiments using two independent RNA samples are shown. The mean expression ratios from the corresponding microarray data are also indicated.