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Research article

IDENTIFICATION OF ENDOPLASMIC RETICULUM STRESS RESPONSE GENES IN HOMOLOGOUS VS. HETEROLOGOUS ASF INFECTIONS *IN VITRO*

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The endoplasmic reticulum (ER) is crucial for the production, processing and transport of proteins. Infection with pathogens activates Unfolded Protein Response (UPR), which can lead to their survival/replication or elimination from the body. Although little is known about the role of the ER stress response in the pathogenesis of viral infections, the regulation of ER stress may be important in intractable infectious diseases. We conducted a comparative analysis of the expression of genes involved in ER stress response in peripheral blood mononuclear cells (PBMCs) from animals immunized with an attenuated strain of ASFV strain Congo-a (KK262) and then stimulated *in vitro* by two serologically different virulent strains Congo-v (K49) or Mozambique-v (M78), to expand our understanding of the early determinants of response to homologous and heterologous infection. We found up-regulation of genes of all three sensory molecules (PERK, ATF6 and IRE1) of UPR pathway in cells infected with only a homologous strain. For the first time, a number of up-regulated genes of the ER-associated degradation pathway (ERAD), which destroys misfolded proteins, were also detected. By understanding how viruses modify elements of cellular response to stress, we learn more about the pathogenesis, as well as how we can use it to prevent viral diseases.

Keywords: African swine fever virus, transcriptome, endoplasmic reticulum stress, endoplasmic reticulum-associated degradation pathway

INTRODUCTION

African swine fever (ASF) is an acute viral hemorrhagic disease of domestic pigs and wild boars with a mortality rate close to 100% [1]. After being imported from East Africa to Georgia, ASFV has been circulating in Eastern Europe since 2007, in the European Union since 2014 and in Asia since 2018 [2]. The spread of the disease outside Africa has become a global threat with enormous economic losses for pig raising countries [3]. There is no vaccine against ASF, although it has been

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reported that animals immunized with an attenuated ASFV strain were protected from challenge by a homologous virulent virus of the same serogroup [4-7]. However, such protection was not observed in the case of challenge by a heterologous virulent virus of another serotype [8,9].

ASFV is a member of the group of nucleocytoplasmic large DNA viruses (NCLDVs) [10]. These viruses replicate their DNA in the cytoplasm of infected cells and produce massive membrane rearrangements. During infection, viruses induce remodeling of cell membranes to generate their replication factories (RFs), assembly and release of virions. Moreover, these membranes can protect viral nucleic acids and proteins from exposure to the host immune system [11]. The mechanism of formation of the inner membrane of the virions is still under debate [11]. The current model assumes that the formation of the crescent membrane (the precursor of the inner membrane of the virion) is derived from the fragmented endoplasmic reticulum (ER) [12]. The study of ASFV assembly revealed the same mechanism associated with ER rupture [13].

ER is an intracellular organelle which forms a network from the nuclear envelope to the plasma membrane using a complex system of membrane sheets and tubules [14]. Sheets (so called rough ER) coated with a large number of ribosomes carry out the synthesis, folding and post-translational modification of membrane and secreted proteins. On the other hand, tubules (smooth ER) are considered as storage for calcium, as well as for lipid synthesis. The structure of the ER is dynamic and can quickly adapt to changes in the state of cells in response to physiological or pathological stimuli [15]. In order to maintain control over the quality of synthesized proteins and ensure that only properly folded proteins are secreted, there are three different pathways in the ER called the unfolded protein response (UPR). This system is activated when misfolded proteins accumulate or calcium leaks from the membranes. The three signaling pathways consist of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6) [16]. Normally, all three transmembrane proteins (PERK, IRE1 and ATF6) are in their inactive form by binding to the chaperone BiP. In addition to protein folding promotion, BiP is able to export misfolded proteins towards ER-associated protein degradation (ERAD). When misfolded proteins accumulate in the ER, BiP dissociates from PERK, IRE1 and ATF6 and leads to their activation. PERK and IRE1 switch from a monomeric conformation to a homodimeric active state. ATF6 becomes active after its cleavage and translocates into the nucleus, activating the transcription of genes responding to ER-stress [17]. Activation of the UPR system leads to overcoming ER stress. If UPR fails to restore ER homeostasis, apoptosis is induced.

Infection with pathogens activates UPR, which can lead to their survival/replication or elimination from the body, since ER stress is associated with autophagy or apoptosis. Although little is known about the role of the ER-stress response in the pathogenesis of viral infections, the regulation of ER-stress may be important in intractable infectious diseases. Viruses can interact with the host's UPR in order to create an

environment favorable for infection and avoid the host's immune response. Indeed, it is reported that ER-stress and UPR activation do not protect against infection with reoviruses and hepatitis B virus, but rather contribute to their replication [18, 19].

Although the ASF virus uses the ER for its reproduction, very little is known about the induction of UPR during infection. It has been shown that ASFV activates the ATF6 branch of UPR, thereby contributing to infection [20]. In another study, it was shown that ASFV, on the contrary, inhibits the activation of the CHOP of ATF6 branch of UPR [21]. It should be noted that both of these studies were performed in vitro on Vero cells infected with an adapted strain of the ASF virus BA71. The study of individual viral proteins revealed that the structural protein p17 of the ASFV induces the production of ER-stress-reactive oxygen species that inhibit cell proliferation [22]. Another protein, K205R, is able to trigger ER-stress by activation of ATF6 and PERK pathways [23]. Whether there are other ASF virus proteins that modulate ER stress remains unknown.

Analysis of transcriptome data allows tracking changes in the expression of all genes simultaneously during infection and detecting activation or inhibition of numerous biological pathways. In our previous paper, we described a comparative analysis of PBMCs of animals immunized with an attenuated ASFV strain, and then stimulated in vitro by two serologically different virulent strains to expand our understanding of the early determinants of response to homologous and heterologous infection [24]. Earlier we discussed the expression of genes involved in the immune response, such as cytokines and chemokines, interferons and interferon-stimulated genes. However, our results showed that not only genes of the immune response, but also genes involved in endocytosis/phagocytosis and cellular stress response may be important for the formation of cross-protective immunity. Here we analyze changes in the expression of genes and pathways involved in ER-stress response.

MATERIALS AND METHODS

Cell Cultures and Viruses

Attenuated ASFV Congo-a strain (KK262, Genotype I, Serogroup 2), parent virulent ASFV Congo-v strain (K49, Genotype I, Serogroup 2) and virulent ASFV Mozambique-v strain (M78, Genotype V, Serogroup 3) were received from the reference collection of the Federal Research Centre for Virology and Microbiology, Russia. Strain K49 was originally isolated in 1949 from a domestic pig (*Sus scrofa domesticus*) in Katanga province of the Democratic Republic of the Congo [25]. The ASFV strain KK262 is a derivative of the highly virulent strain K49, obtained as a result of 50 consecutive passages in pig kidney cell lines and 262 passages in pig bone marrow cell culture [26]. Strain M78 was originally isolated in Mozambique and transferred to the Federal Research Center for Virology and Microbiology in 1978, but the exact date of the outbreak is unknown.

PBMCs were prepared from defibrinated blood using the lymphocyte separation media (Gibco) as described earlier [24].

Virus titration was performed on 96-well plates by visualizing of CPE in PBMCS of pigs. Titers were expressed as means of tissue culture infectious dose ($TCID_{50}$) according to the Reed–Muench method [27].

ASFV infection of PBMCs

PBMCs were prepared from animals immunized with ASFV Congo-a in the experiment described earlier [28]. Briefly, pigs were infected intramuscularly with 10^6 $TCID_{50}$ of Congo-a virus. At day 21 post-infection (dpi), the animals were boosted with the same dose of the same virus. Three weeks later (42 dpi.), PBMCs were isolated from defibrinated blood using the Lymphocyte separation media (Gibco). The cells were inoculated with two different virulent viruses with the multiplicity of infection of 1 (MOI=1). 5 hours after inoculation with the virus, PBMCs were washed once with sterile PBS and used to isolate total RNA [24].

To identify the genome of the ASFV, PCR of the B646L gene was performed in accordance with the protocol published by King et al. [29]. PCR of the β-actin gene was used as endogenous control. PCR reactions were carried out on a CFX96TM thermal cycler (Bio-Rad, Hercules, CA, USA).

RNA extraction and sequencing

The total RNA was isolated with Trisol LS reagent and PureLink RNA Micro Scale Kit (Invitrogen) according to manufacturer's instructions. Analysis of the quality of the obtained RNA was carried out on the Bioanalyzer 2100 using RNA 6000 Nano Kit (Agilent Technologies) according to the recommendations of the manufacturer. PolyA RNA was purified with Dynabeads® mRNA Purification Kit (Ambion). Illumina library was made from polyA NEBNext® Ultra™ II RNA Library Prep (NEB) according to manual. Sequencing was performed on HiSeq1500 system with 50 bp read length. At least 10 million of reads were generated for each sample. Reads were aligned with the porcine genome using STAR aligner and differentially expressed transcripts were count by DESeq2.0 [30].

Statistical and bioinformatic analyses

Log2 fold changes in signal intensity were applied in statistical analysis to identify differentially expressed genes (DEGs). The P-values were calculated using R software DESeq2.0. To account for multiple testing, the p-values were adjusted using the Benjamini and Hochberg method, and the false discovery rate (FDR)-corrected P-value were calculated. Differences in gene expression with a FDR-value of 0.05 or less and an expression difference of 50% or more were considered as DEGs. The genes up- or down-regulated were expressed as positive and negative values (fold),

respectively. The identified DEGs were compared with human reference genes. The bioinformatics program (DAVID Bioinformatics Resources 6.8) was used to identify biological pathways (GOTERM_BP_DIRECT, REACTOME_PATHWAY and KEGG_PATHWAY) for significantly different DEGs.

RESULTS

Pathway analysis of genes involved in the cellular response to stress.

A detailed analysis of the DEGs involved in the stress response using Reactome database (https://reactome.org) revealed the 7 most significant pathways (Table 1). These pathways were up-regulated in cells infected with a homologous strain, but not in PBMCs infected with a heterologous strain. Over-expressed pathways include the ATF6, PERK, IRE1/XBP1 and ATF4 signaling pathways, as well as unfolded protein response (UPR). All of them are involved in the endoplasmic reticulum response to stress and are supposed to induce the expression of chaperone genes.

Table 1. Biological pathways of cellular response to stress with up-regulated genes in PBMCs infected with strain K49 compared to infected with strain M78 (FDR ≤0.05)

Pathway name	Entities				Reactions	
	found	ratio	p-value	FDR*	found	ratio
ATF6 (ATF6-alpha) activates chaperone genes	8/15	$9.9x10^{-4}$	$1.1x10^{-16}$	$1.4x10^{-15}$	5/5	$3.6x10^{-4}$
PERK regulates gene expression	11/42	$0.3\ x10^{-2}$	$1.1x10^{-16}$	$1.4x10^{-15}$	11/11	$7.9x10^{-4}$
IRE1alpha activates chaperones	14/101	$0.7\ x10^{-2}$	$1.1x10^{-16}$	$1.4x10^{-15}$	50/53	$0.4x10^{-2}$
Unfolded Protein Response (UPR)	28/155	$0.1\ x10^{-1}$	$1.1x10^{-16}$	$1.4x10^{-15}$	78/94	$0.7x10^{-2}$
ATF6 (ATF6-alpha) activates chaperones	8/17	$0.1\ x10^{-2}$	$3.3x10^{-16}$	$3.7x10^{-15}$	10/10	$7.3x10^{-4}$
XBP1(S) activates chaperone genes	11/95	$0.6\ x10^{-2}$	$2.2x10^{-15}$	$2.2x10^{-14}$	47/47	$0.3x10^{-2}$
ATF4 activates genes in response to endoplasmic reticulum stress	8/34	$0.2\ x10^{-2}$	$7.3x10^{-14}$	$6.5x10^{-13}$	7/7	$5.1x10^{-4}$

Expression of genes involved in the cellular response to stress

A comparison of biological processes with differently expressed genes (DEGs) in cells infected with homologous or heterologous ASFV strains showed that the most significant difference was associated with the response to cellular stress [24]. Twenty two genes involved in this response were up-regulated in PBMCs infected with the K49 ASFV strain and not altered in cells infected with the M78 strain (Table 2). A schematic overview of UPR gene activation is shown in Figure 1 and 2. Although the difference in the expression of most genes varied from 1.5 times (DERL1) to 3.2 times (SGK1), but for two genes ASNS and TRIB3, this difference reached 5.1 times and 8.7 times, respectively. The study of the cellular response to various stimuli by

transcriptomic analysis makes it possible to detect changes in transcription by 1.5-3 times, which is difficult when using real-time PCR. Moreover, such a comprehensive analysis makes it possible to determine not only changes in the expression of individual genes, but also to trace changes in numerous signaling pathways.

Table 2. Differently expressed genes of cellular stress response in PBMCS infected with ASFV K49 strain compared to M78 strain (FDR ≤0.05)

Gene name	Base Mean	K49vsM78		K49vsNeg		M78vsNeg	
		Fold	padj	Fold	padj	Fold	padj
ASNS	589	5,1	$2{,}40\times10^{-66}$	6,5	$1{,}88\times10^{-77}$	1,3	$3{,}17\times10^{-01}$
ATF4	6933	2,4	$4{,}47\times10^{-63}$	2,7	$2{,}75\times10^{-86}$	1,1	$1{,}97\times10^{-02}$
ATF6	732	1,7	$2{,}45\times10^{-08}$	1,5	$5{,}48\times10^{-07}$	0,9	$7{,}86\times10^{-01}$
CREB3L1 (OASIS)	35	2,8	$6{,}32\times10^{-03}$	2,5	$1{,}02\times10^{-02}$	0,9	$9{,}57\times10^{-01}$
CREB3L2 (Tisp40)	243	2,0	$2{,}29\times10^{-07}$	2,4	$1{,}24\times10^{-10}$	1,2	$6{,}61\times10^{-01}$
DDIT3 (CHOP)	263	2,9	$1{,}21\times10^{-16}$	3,0	$8{,}27\times10^{-19}$	1,0	$9{,}67\times10^{-01}$
DERL1	1227	1,5	$1{,}88\times10^{-08}$	1,5	$1{,}95\times10^{-07}$	1,0	$8{,}66\times10^{-01}$
DNAJB9	492	2,5	$1{,}48\times10^{-19}$	3,3	$3{,}55\times10^{-33}$	1,4	$4{,}44\times10^{-02}$
EDEM1	3971	1,8	$7{,}23\times10^{-28}$	2,3	$3{,}05\times10^{-57}$	1,3	$2{,}80\times10^{-06}$
EIF2AK3 (PERK)	678	2,5	$1{,}11\times10^{-24}$	2,4	$1{,}14\times10^{-23}$	0,9	$8{,}70\times10^{-01}$
ERN1 (IRE1)	162	2,4	$6{,}14\times10^{-08}$	2,4	$5{,}15\times10^{-08}$	1,0	$9{,}83\times10^{-01}$
HERPUD1 (HERP)	1494	2,3	$1{,}43\times10^{-39}$	3,0	$2{,}60\times10^{-66}$	1,3	$1{,}75\times10^{-03}$
HSPA5 (BiP)	14238	2,4	$1{,}02\times10^{-70}$	3,2	$1{,}53\times10^{-132}$	1,3	$6{,}13\times10^{-10}$
PPP1R15A (GADD34)	2780	2,3	$2{,}32\times10^{-43}$	2,9	$7{,}25\times10^{-82}$	1,3	$4{,}07\times10^{-05}$
PDIA4	2968	2,2	$3{,}89\times10^{-44}$	2,6	$5{,}54\times10^{-66}$	1,2	$1{,}5\times10^{-02}$
SEL1L	1233	2,4	$4{,}53\times10^{-28}$	2,4	$6{,}51\times10^{-29}$	1,0	$9{,}46\times10^{-01}$
SELENOS	605	1,8	$1{,}45\times10^{-10}$	2,4	$5{,}29\times10^{-23}$	1,4	$6{,}26\times10^{-03}$
SYVN1 (HRD1)	133	1,7	$8{,}79\times10^{-03}$	1,4	$6{,}70\times10^{-02}$	0,9	$7{,}38\times10^{-01}$
TRIB3	386	8,7	$7{,}90\times10^{-67}$	9,6	$3{,}18\times10^{-79}$	1,1	$9{,}09\times10^{-01}$
WFS1	108	2,3	$3{,}66\times10^{-05}$	2,0	$3{,}04\times10^{-04}$	0,9	$8{,}70\times10^{-01}$
XBP1	1092	1,6	$2{,}87\times10^{-10}$	2,4	$3{,}95\times10^{-30}$	1,5	$1{,}09\times10^{-06}$
SGK1	2234	3,2	$4{,}98\times10^{-80}$	2,0	$5{,}02\times10^{-36}$	0,6	$2{,}32\times10^{-13}$

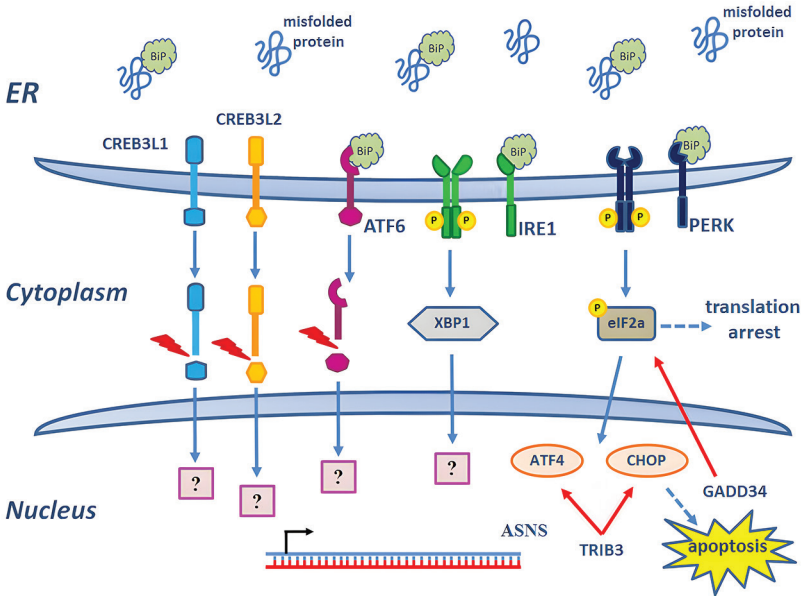

Figure 1. Schematic overview of UPR activation during infection of cells from pigs immunized with an attenuated strain and then infected with the same virulent strain. Three ER stress sensors (IRE1, PERK and ATF6) are activated at an early stage of infection. This activates transcription factors ATF4 and CHOP and, consequently, over-expression of their target genes (ASNS, GADD34, TRIB3). Synthesis of TRIB3 can provide a negative feedback of activation of the PERK pathway, which promotes cell survival. Over-expression of ER-resident transcription factors CREB3L1 and CREB3L2 can prevent the accumulation of unfolded (viral) proteins.

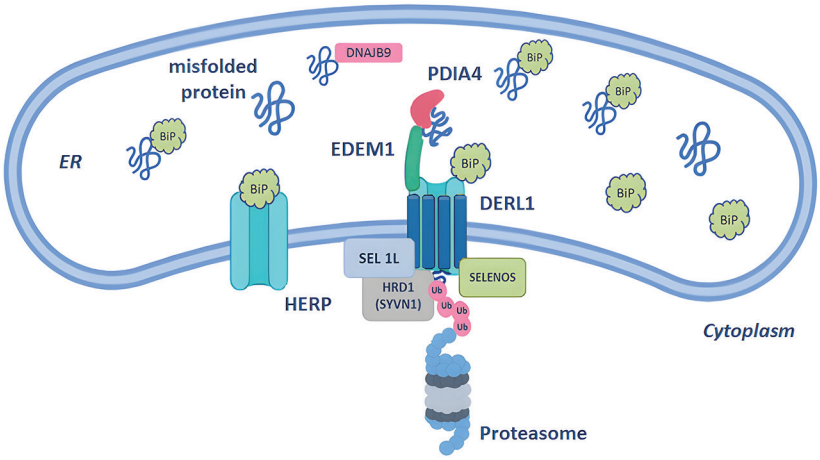

Figure 2. Schematic overview of ERAD activation during infection of cells from pigs immunized with an attenuated strain and then infected with same virulent strain. ER-proteins (EDEM1, DNAJB9, PDIA4) recognize ERAD substrates, which results in the assembly of a retrotranslocon (HERP/BiP comlex). HERP facilitate the oligomerization of the HRD1 E3 Ligase and the formation of translocon containing Derlin (DERL1), HRD1 and SEL1L. SELENOS binds to DERL1 and mediates the retrotranslocation of misfolded proteins into the cytosol. Retrotranslocation exposes ERAD substrates for ubiquitination and proteasomal degradation [53].

DISCUSSION

Cellular stress caused by abnormal accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER) becomes one of the possible causes of human and animal diseases. The ER proteostasis is regulated by the unfolded protein response (UPR), a signal transduction pathway that recognizes the accuracy of protein folding in the ER lumen. UPR transmits information about the state of protein folding to the nucleus and cytosol to regulate the protein folding or, in the case of chronic damage, induce apoptosis. UPR activation affects almost every aspect of the secretory pathway, altering the rate of protein synthesis, protein folding and maturation, protein transport and elimination of misfolded proteins through autophagy and ER-associated protein degradation (ERAD) pathway [31]. The basic UPR pathways in mammals consist of three main signaling cascades initiated by transmembrane ER proteins: PERK, IRE1α and ATF6 (Fig.1) Activation of PERK induces phosphorylation of eIF2a and protein translation arrest, as well as activation of transcription factors ATF4 and C/EBP Homologous Protein (CHOP) [32]. CHOP is known to promote apoptosis [33]. GADD34, the CHOP transcription target, induces dephosphorylation of eIF2a, leading to the restoration of protein translation [34]. IRE1 is a kinase with a unique ability to stimulate IRE1-dependent mRNA decay (RID) to release a stressed ER from the burden of new proteins [35,36]. In addition, IRE1 induces unconventional splicing of its main substrate XBP1. This processing event leads to the expression of the active transcription factor XBP1, which up-regulates genes involved in translocation, folding and secretion of ER proteins, as well as degradation of misfolded proteins [37]. Another way of the ER reaction to stress is to activate the ATF6 protein, which passes from the ER into the Golgi apparatus, where it is cleaved with the release of a fragment containing a transcription factor (Fig.1). This factor translocates into the nucleus, inducing the expression of target genes [38,39]. ATF6 and XBP1s act in overlapping pathways, regulating the transcription of genes encoding ER chaperones and enzymes that promote ER protein translocation, folding, maturation and secretion, as well as degradation of misfolded proteins [40-42].

Comparison of differently expressed genes (DEGs) in PBMCs of animals immunized with an attenuated ASFV strain, and then stimulated in vitro by two serologically different virulent strains revealed early activation of transcription of genes involved in the signaling pathways of the ER stress response. Unexpectedly, this activation was detected only in cells infected with a homologous ASFV strain, whereas no changes in the expression of these genes were observed in cells infected with a heterologous strain (Table 2). Most likely, when viral proteins accumulate during infection with a heterologous strain, UPR is also triggered, but cells that have already encountered a homologous virus initiate this response earlier. Although up-regulation of transcription was detected for all three sensory molecules (PERK, ATF6 and IRE1), activation of transcription of downstream target genes in the ATF6 and IRE1 pathways was not detected 5 hpi. In addition, there was only a slight increase in XBP1 transcription, both

in cells infected with a homologous strain and in cells infected with a heterologous strain (Table 2).

Most viruses use ER to create replication factories and cause a remodeling of the structure and function of ER [11]. Activation of UPR can be proviral, antiviral or pathogenic, depending on the specific type of infection [32]. Thus, it is reported that ER-stress and UPR activation do not protect cells from infection with reovirus and hepatitis B virus, but, on the contrary, contribute to their replication [18,19]. Flaviviruses, including Dengue and Zika, also activate UPR by up-regulation of the ATF6 and IRE1/XBP1s signaling pathways [43]. Porcine circovirus type 2 (PCV2) triggers the eIF2a-ATF4–CHOP pathway and caspase activation to enhance its replication in cells [44]. Regulation of eIF2a phosphorylation has been shown to be important for the survival of enveloped viruses, such as herpes simplex virus (HSV) [45]. Other studies have shown that the PERK pathway is important for antiviral protection of cells from vesicular stomatitis virus [46]. West Nile virus and Coxsackie virus B3 induce ER-stress-mediated apoptosis by stimulating CHOP synthesis [47,48]. It is reported that IRE1 is necessary for the induction of autophagy when infected with infectious bronchitis virus [49].

The up-regulation of the PERK signaling pathway leads to phosphorylation of eIF2a and the arrest of protein translation, as well as to the activation of transcription factors ATF4 and CHOP (Fig.1). Consequently, transcripts initiated by these factors accumulate. Indeed, we found increased transcription of target genes such as asparagine synthetase (ASNS), tribbles homolog 3 (TRIB3) and protein phosphatase 1 regulatory subunit 15A (GADD34), as well as increased transcription of ATF4 and CHOP themselves (Table 2, Fig.1). ASNS is the most important enzyme involved in the synthesis of asparagine, and can affect the replication of various viruses [50]. Over-expression of GADD34 leads to dephosphorylation of eIF2a and restoration of translation. Up-regulation of TRIB3, a protein kinase that acts as a stress response regulator suppressing CHOP-dependent cell death during ER stress and suppressing ATF4 function (Fig.1) [51]. Therefore, over-expression of GADD34 and TRIB3 is a negative feedback for the PERK1 pathway, promoting cell survival. This may explain why induction of CHOP and ATF4 was not detected at the late stage of ASFV infection of cells [21].

Up-regulation of two ER-resident transcription factors CREB3L1 and CREB3L2 was also detected in cells infected with a homologous ASFV strain (Table 2, Fig.1). These factors exist as membrane-bound precursors and are activated by proteolysis in response to ER stress [52, 53]. CREB3L1 has been shown to inhibit the proliferation of cells infected with mouse herpes virus 68, HCV, West Nile virus (WNV) and Sendai virus [52]. It has been shown for CREB3L2 that it is an ER stress transducer and plays an important role in preventing the accumulation of unfolded proteins [53]. Also, over-expression of CREB3L2 suppresses ER-stress-induced cell death [53]. Therefore, over-expression of CREB3L1 and CREB3L2 may be one of the ways of the cell that prevent the reproduction of the ASF virus.

Another mechanism that degrades misfolded proteins is the ER-associated degradation (ERAD) pathway [54]. ER chaperones detect misfolded proteins in the ER lumen and transfer them to ERAD adapters on ER membrane, and then they are released into the cytosol (Fig.2). Misfolded proteins undergo proteolytic degradation by the ubiquitin-proteasome system [55]. A number of up-regulated genes of the ERAD pathway were detected in cells infected with a homologous ASFV strain (Table 2). Among them are genes encoding DERL1, DNAJB1, SELENOS, forming protein complex necessary for the recognition of misfolded proteins and their translocation from ER to cytosol [56-58]. Also, an increased level of transcription was found for genes encoding EDEM1 and SEL1L (Table 2). It has been reported that EDEM1 specifically binds unfolded proteins in association with the ER membrane adapter protein SEL1L [59]. Degradation of proteins by proteasomes required their polyubiquitination, and we also found up-regulation of genes encoding SYVN1 (E3 ubiquitin ligase, which accept ubiquitin from a specific ER E2 ligase and transfer it to the substrate) and HERPUD1 (ubiquitin domain protein) [59,60]. The proteins SYVN1, HERPUD1 and DERL1 form a high molecular mass protein complex associated with the ER membrane that facilitates ERAD.

Viruses can use the ERAD process to facilitate their replication or to manipulate the immune response [54]. One of the best studied examples is the ability of the herpesvirus to induce degradation of major histocompatibility complex class I (MHC-I) by viral proteins US2 and US11 [61,62]. Human immunodeficiency virus type 1 decreases the levels of both CD4 mRNA and protein to avoid T-cell activation [63]. Some viruses, such as hepatitis virus B (HBV) or C (HCV), use ERAD to reduce the amount of glycoproteins by inducing the expression of EDEM proteins [54]. An increased level of EDEM leads to degradation of HCV Env protein and, consequently, to a decrease in the number of viral particles [64]. This avoids the activation of innate and adaptive immunity, which leads to chronic infection [64,65]. On the other hand, early activation of the ERAD system may be beneficial for cell survival. Thus, it has been shown that viral envelope glycoprotein gO of human cytomegalovirus (HCMV) is constitutively degraded during infection by ERAD pathway [66].

It has been shown that that ASFV or its proteins are capable of triggering an ER-stress response via the ATF6 or PERK pathways [20-23], but for the first time we detected the up-regulation of genes involved in the ERAD pathway. Since there are too many different proteins involved in the UPR and ERAD pathways, transcriptome analysis revealed possible contributors to ASFV infection. It remains unclear whether the launch of UPR and ERAD is an advantage for ASF virus replication or vice versa for the survival of host cells. It is believed that vaccination of animals with an attenuated ASFV strain leads to the protection of these animals from infection with a virulent homologous strain [25]. Early activation of the ER-stress response can indeed contribute to cell survival and lead to degradation of viral proteins and particles, as well as the absence of clinical signs in animals. However, such an active decrease in the viral load on cells may, on the contrary, lead to the formation of a chronic infection,

the signs of which may appear a long time after infection. By understanding how viruses modify cell stress response elements, we learn more about the cellular process, as well as how we can use it to prevent viral diseases.

CONCLUSIONS

Accumulation of misfolded proteins in the ER leads to activation of UPR and/or ERAD pathways to restore ER homeostasis. When the capacity of UPR and ERAD is exceeded, excessive ER stress can eventually lead to apoptosis. Although the mechanisms of ER-stress response have been extensively studied, it is unclear how this response regulates both apoptotic and adaptive pathways. Viruses can use the UPR and ERAD pathways to facilitate their replication or to manipulate the immune response. ASFV can induce both UPR and ERAD signaling pathways; however, it remains unclear whether this is a protective mechanism of the cell against viral infection, or a way for the virus to avoid cellular and immune responses.

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Authors' contributions

NK conceptualization, methodology, design of the study, executed the study, visualization, wrote the first draft of the manuscript, reviewed and edited the manuscript. AK methodology, executed the study, wrote sections of the manuscript, reviewed and edited the manuscript. NV reviewed and edited the manuscript. GK conceptualization, design of the study, executed the study, visualization, wrote sections of the manuscript, reviewed and edited the manuscript, exercised project administration. All authors have read and agreed to the published version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The animal study was reviewed and approved by the Research Ethics Committee of the Federal Research Center of Virology and Microbiology, Russia (No 3/2021).

Data Availability Statement

The data presented in the study are deposited in the https://www.ncbi.nlm.nih.gov/sra/PRJNA860828.

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IDENTIFIKACIJA GENA ODGOVORA NA STRES ENDOPLAZMSKOG RETIKULUMA U HOMOLOGNIM NASUPROT HETEROLOGNIM INFEKCIJAMA AKS *IN VITRO*

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Endoplazmatski retikulum (ER) je ključan za sintezu, obradu i transport proteina. Infekcija sa patogenima aktivira Unfolded Protein Response (UPR, odgovor razmotanih proteina), što može dovesti do njihovog preživljavanja/replikacije ili eliminacije iz tela. Iako se malo zna o ulozi odgovora na stres ER u patogenezi virusnih infekcija, regulacija stresa ER može biti važna kod nerešivih infektivnih bolesti. Sproveli smo komparativnu analizu ekspresije gena uključenih u odgovor ER na stres u mononuklearnim ćelijama periferne krvi (PBMC) od životinja imunizovanih oslabljenim sojem ASFV soja Congo-a (KK262), a zatim stimulisanih in vitro sa dva serološki različita virulentna soja, Kongo-v (K49) ili Mozambik-v (M78), kako bismo proširili naše razumevanje ranih determinanti odgovora na homologlognu i heterologlognu infekciju. Pronašli smo pojačanu regulaciju gena sva tri senzorna molekula (PERK, ATF6 i IRE1) UPR puta u ćelijama inficiranim samo homologlognim sojem.Po prvi put, otkriven je i određeni broj naviše regulisanog gena puta degradacije povezanog sa ER (ERAD), koji uništava pogrešno savijene proteine. Razumevanjem kako virusi modifikuju elemente ćelijskog odgovora na stres, saznajemo više o patogenezi, kao i o tome kako je možemo koristiti za sprečavanje virusnih bolesti.