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Evolution of African swine fever virus genes related to evasion of host immune response

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ABSTRACT

African swine fever (ASF) is a notifiable and one of the most complex and devastating infectious disease of pigs, wild boars and other representatives of *Suidae* family. African swine fever virus (ASFV) developed various molecular mechanisms to evade host immune response including alteration of interferon production by multigene family protein (MGF505-2R), inhibition of NF-κB and nuclear activating factor in T-cells by the A238L protein, or modulation of host defense by CD2v lectin-like protein encoded by EP402R and EP153R genes. The current situation concerning ASF in Poland seems to be stable in comparison to other eastern European countries but up-to-date in total 106 ASF cases in wild boar and 5 outbreaks in pigs were identified. The presented study aimed to reveal and summarize the genetic variability of genes related to inhibition or modulation of infected host response among 67 field ASF isolates collected from wild boar and pigs. The nucleotide sequences derived from the analysed A238L and EP153R regions showed 100% identity. However, minor but remarkable genetic diversity was found within EP402R and MGF505-2R genes suggesting slow molecular evolution of circulating ASFV isolates and the important role of this gene in modulation of interferon I production and hemadsorption phenomenon. The obtained nucleotide sequences of Polish ASFV isolates were closely related to Georgia 2007/1 and Odintsovo 02/14 isolates suggesting their common Caucasian origin. In the case of EP402R and partially in MGF505-2R gene the identified genetic variability was related to spatio-temporal occurrence of particular cases and outbreaks what may facilitate evolution tracing of ASFV isolates. This is the first report indicating identification of genetic variability within the genes related to evasion of host immune system which may be used to trace the direction of ASFV isolates molecular evolution.

1. Introduction

African swine fever is a contagious disease of pigs, wild boars as well as other hosts belonging to the Suidae family (Costard et al., 2013; OIE, 2012). The virus also replicates in soft ticks belonging to *Ornithodoros* genus which represents one of the important vector of its spread (Diaz et al., 2012). African swine fever virus (ASFV) belongs to the *Asfivirus* genus of *Asfarviridae* family and as other large DNA viruses developed mechanisms to evade or inhibit host immune response (Afonso et al., 2004; Chapman et al., 2008; Correia et al., 2013; Dixon et al., 2004; Zsak et al., 2001). Currently, no vaccine against ASF is available and it might be predicted that the vaccine will not be accessible for at least a few coming years (Zakaryan and Revilla, 2016). In domestic pigs ASFV causes acute and fatal infection whereas in European wild boars this process might be acute, subclinical or chronic without any disease signs (Colgrove et al., 1969; Costard et al., 2013; Penrith and Vosloo, 2009; Sánchez-Vizcaíno et al., 2013, 2015). Previous studies showed that ASFV may persist in natural hosts for a long time period after animal recovery suggesting the specific viral mechanisms which are used to evade host immune response. The viral exploitation of molecular machinery of the cell is mediated by particular proteins encoded by the genes taking part in numerous cellular processes or mimicking factors regulating immediate or

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late transcription factors (Afonso et al., 2004; Correia et al., 2013). Up to date at least several genes of ASFV are known to influence the immune response of the host. These include A238L, A179L, EP153, A224L or EP402R (Dixon et al., 2004, 2013; Tulman et al., 2009; Zsak et al., 2001). Indeed, it has been previously shown the domain of A238L gene has a homology to IκBα factor and may interact with p65 of NFκB factors which take part in transcription activation (Powell et al., 1996; Revilla et al., 1998). It has also been found that another domain of A238L may interact with calcineurin phosphatase (CanPase or protein phosphatase 3) as an inhibitor of nuclear factor in T-cells (NFAT) (Miskin et al., 2000). The mutations possible in phosphorylation sites of A238L may lead to its inactivity in some ASFV isolates.

The next important factor related to the inhibition mechanism of host defense system is represented by the gene encoded by open reading frame EP153R of ASFV. This region is responsible for apoptosis control in the ASFV infected macrophages (Hurtado et al., 2011). Comparison of nucleotide sequence of this region with databases showed that it might be homologous to a particular type of C-lectin molecules such as CD69, Ly49A or CD94. It has been shown that the dimer or EP153R may interact with MHC-I antigens through the NKG2D and ULBP3 ligands (Galindo et al., 2000). EP153R does not influence the synthesis or maturation of MHC-I molecules but inhibits cellular transport of this molecule from endoplasmic reticulum to cell membrane. The evidence on the role of EP153R was also shown after transfection of small interfering RNA into the cells infected with ASFV or the study on virus recombinants lacking EP153R (Hurtado et al., 2011).

The third ASFV genome region important for the modulation of interferon production is associated with multigene family genes (MGF). It has been shown that low virulent ASFV isolates are lacking different number of copies of these genes belonging to MGF360 or MGF505 families (O'Donnell et al., 2015). Experimental deletion of these genes within ASFV genome resulted in increased interferon type-I production suggesting their important role in its level modulation (Afonso et al., 2004; De la Vega et al., 1990; Dixon et al., 2004; Zsak et al., 2001).

The fourth but not the last important EP402R gene is directly responsible for hemadsorption of erythrocytes since its deletion abrogated this phenomenon (Dixon et al., 2004). The role of hemadsorption is still unknown but at least it has been shown the erythrocytes bind to the surfaces of organs in ASF infected pigs (Malmquist and Hay, 1960; Vallée et al., 2001).

ASFV isolates may differ in virulence and their ability of evading host immune machinery (Muangkram et al., 2015; Portugal et al., 2015). The virus isolates circulating for the last 24-months in Poland present interesting opportunity to compare possible evolution direction on the basis of genes related to inhibition of cellular factors related to immune response. The goal of this study was to summarise the genetic variability within the ASFV genes related to evasion of host immune response. These data have been combined with spatio-temporal background of ASFV cases and outbreaks. It is well known that the time of wild boar carcasses collection might be independent from the date of virus infection and animal death but the presented study might be hepful to trace the direction of ASFV molecular evolution.

2. Materials and methods

2.1. Strains and controls

The examined samples included reference ASFV strain Ba71V and field isolates represented by 64 cases in wild boars and 3 outbreaks in pigs that occurred in Poland between February 2014 and November 2015. The Vero-adapted ASFV strain Ba71V used as positive control was obtained thanks to courtesy of the European Union Reference Laboratory for ASF (CISA-INIA, Valdeolmos, Spain).

2.2. DNA extraction

DNA from the infected cell cultures as well as reference virus stock were extracted using High Pure PCR Template Preparation Kit, following the manufacturer's procedure (Roche Diagnostics, Basel, Switzerland). DNA from the whole blood and tissue homogenates from infected animals organs was extracted accordingly to the manufacturer's conditions and procedures. The final elution volume was 50 μl of sterile nuclease-free water. The extracted DNA was stored at −20 °C until further analysis.

2.3. PCR primers for amplification and sequencing

PCR primers complementary to the A238L, EP153R, EP402R and MGF505-2R sequences genes were designed using Primer 3 software (http://primer3.wi.mit.edu). The primers were designed on the basis of the complete genome sequence of ASFV-isolate Georgia 2007/1 (GenBank accession number: FR682468.1). The obtained primer sequences were verified with GenBank database using BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST) to ensure their specificity to target sequences. The primer sequences were listed in Table 1.

2.4. PCR amplification for sequencing

PCR was performed in 25 μl final volume with entire reaction conditions set up for each pairs of primers individually, accordingly to the manufacturer's recommendations of MyTaq™ HS DNA Polymerase (Bioline, Gdansk, Poland). The reaction mixture contained: 12.5 μl of MyTaq™ HS DNA Polymerase, 9.5 μl PCR-grade water, 1 μl of each primer and 1 μl (~200 ng) template DNA.

Table 1
Primer sequences complementary to ASFV genome region related to evading host immune response. The PCR annealing temperature for each primer pair has been provided. Concentration of each primer was 5 pM per reaction.

Primer name	Sequence (5′-3′)	Annealing temperature (°C)	Localisation within the genome of Georgia 2007/1 strain (Genbank accession number: FR682468.1)
A238L-F	GAGATTACTTTCCATACTTG	45.6	50441–50460
A238L-R	AGGAGTAAAATCATATAGTG	45.6	51181–51200
EP153R-F	ATATAACACAAAATTAATC	46.2	72804–72822; 144363–144380
EP153R-R	CATATGTTTTATAATATAGT	45.2	73341–73360
EP402R-F	ACTATATTATAAAACATATG	46.5	73341–73360
EP402R-R	AAGGTTAAATAATTAATATA	46.5	74461–74480
MGF505-2R-F	GCAGAGGTATGATGTCCTTA	48.0	33634–33653
MGF505-2R-R	TTCCTGTTGAACAAGTATCT	48.0	34925–34944