ORIGINAL ARTICLE

Changes in African swine fever virus (*Asfarviridae: Asfivirus: African swine fever virus*) genome associated with adaptation to reproduction in continuous cell culture

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Introduction. African swine fever virus (ASFV) is a large, double-stranded DNA virus in the *Asfarviridae* family. It is the causative agent of African swine fever (ASF). Only the genome of BA71V strain, adapted to Vero cell culture, was fully analyzed.
The **aim** of this study was analyzing the complete genome sequence of two strains of adapted to the growth in CV-1 cell culture (CC) ASFV obtained after 30 and 50 passages, in comparison to the parental virus.
Material and methods. ASFV isolate Odintsovo 02/14 (parental), ASFV adapted variants ASFV/ARRIAH/CV-1/30 and ASFV/ARRIAH/CV-1/50 were all used to extract genomic DNA (gDNA). Sequencing library was constructed using the «Nextera XT DNA library preparation kit» («Illumina», USA).
Results. Genomes of ASFV/ARRIAH/CV-1/30 and ASFV/ARRIAH/CV-1/50 consisted of 186,529 bp and 186,525 bp, respectively. Total 78 single nucleotide polymorphisms (SNPs) were identified between the parental Odintsovo 02/14 and the two high passaged strains, as well as a 2947 bp large-size deletion in the 3' variable region of adapted viruses was detected.
Discussion. ASFV as a DNA-containing virus may not have a very high level of mutation, but this is the second study showing that adaptation to growth in continuous CC leads to large deletions in the genome of the virus.
Conclusion. Mutations in the protein-coding regions of the genome can be synonymous and non-synonymous, i.e. leading to amino acid substitution. Additional research is needed to understand the influence of the mutations described in the adaptation process on the reproduction of the virus and its virulence.

Keywords: *African swine fever virus; complete genome sequencing; adapted viruses; continuous cell culture*

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НАУЧНАЯ СТАТЬЯ

Геномные изменения вируса африканской чумы свиней (*Asfarviridae: Asfivirus: African swine fever virus*), связанные с адаптацией к размножению в перевиваемой культуре клеток

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ORIGINAL RESEARCH

Введение. Возбудитель африканской чумы свиней (*Suidae*) (АЧС) – крупный (175–215 нм) двухцепочечный ДНК-вирус, относящийся к семейству *Asfarviridae*. К настоящему времени секвенирован и подробно проанализирован только геном штамма BA71V, адаптированного к клеточной культуре Vero.
Целью данной работы явился сравнительный анализ полногеномного сиквенса исходного изолята вируса АЧС Odintsovo 02/14 и 2 штаммов, полученных на уровне 30 и 50 пассажей и адаптированных к росту в клеточной культуре CV-1.
Материал и методы. В работе использованы различные варианты возбудителя исходный изолят Odintsovo 02/14 и 2 штамма адаптированного вируса: ASFV/ARRIAH/CV-1/30 и ASFV/ARRIAH/CV-1/50. Библиотеку последовательностей конструировали с использованием набора «Nextera XT DNA library preparation kit» («Illumina», США).
Результаты. Длина геномов штаммов ASFV/ARRIAH/CV-1/30 и ASFV/ARRIAH/CV-1/50 составила 186 529 и 186 525 п.н. соответственно. Всего между исходным и адаптированными вариантами обнаружены 78 однонуклеотидных полиморфизмов (single nucleotide polymorphisms, SNP); кроме того, установлено наличие крупноразмерной делеции величиной 2947 п.н. в правом (3'-концевом) вариабельном регионе у обоих адаптированных штаммов.
Обсуждение. Возбудитель АЧС как ДНК-содержащий вирус может не иметь высокого мутационного статуса. Однако в данном исследовании повторно установлено, что адаптация этого инфекционного агента к росту в перевиваемой культуре клеток (КК) приводит к появлению крупноразмерной делеции в 3'-вариабельной области генома.
Заключение. В связи с недостаточной изученностью данной проблемы необходимо проведение дополнительных исследований, что позволит подтвердить имеющиеся данные относительно влияния каждой из описанных мутаций на характер размножения вируса и степень его вирулентности.

Ключевые слова: *вирус африканской чумы свиней; полногеномное секвенирование; адаптированные вирусы; перевиваемая культура клеток*

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Introduction

African swine fever virus (ASFV) is a large (175–215 nm), double-stranded DNA virus in the *Asfarviridae* family. It is the causative agent of African swine fever (ASF), a haemorrhagic disease with high mortality rates in domestic pigs.

To study the foundations of the pathogenicity and immunogenicity of the ASFV, it is necessary to carry out a comparative analysis of the biological properties of the virus and the structure of its genes. ASFV is able to replicate in porcine primary cell cultures (CC), such as porcine leukocytes CC, alveolar macrophages, etc. However, these cultures are difficult to standardize because their properties differ depending on the characteristics of the animal used, which makes it much more difficult to obtain a genetically uniform sample of virus suitable for analysis. To address this problem, various ASFV isolates have been adapted to grow in continuous cell culture, ex. Georgia 2007/1 and BA71 in Vero cell culture. As a result of this adaptation, ASFV-G/V strain and the BA71V strain were obtained [1, 2]. Although, both these strains were reported to be avirulent following adaptation, only the genome of BA71V was sequenced and analyzed [2].

In the process of adaptation to growth in continuous cell cultures, the virulence of the ASFV decreased [3]. The purpose of this work was a comparative analysis of the nucleotide sequence obtained as a result of the whole genome sequencing of two strains of the ASFV obtained after 30 and 50 passages in the CV-1 cell culture, with the genome of the original isolate.

Previous studies indicated a decrease in virus virulence of isolate ASFV/ARRIAH/CV-1/30, yet the genome of this strain following adaptation was not well characterized [3]. In this study, we investigate how ASFV adaptation to growth in continuous cell culture might influence the virus's genome, and identify the resulting mutations.

Material and methods

Strains ASFV/ARRIAH/CV-1/30 and ASFV/ARRIAH/CV-1/50 were propagated in CV-1 cell culture,

as described previously [4]. Genomic DNA (gDNA) of the ASFVs were extracted using the previously described phenol-chloroform method and the resulting gDNA pellets diluted in nuclease-free water [5]. A sequencing library was constructed using the «Nextera XT DNA library preparation kit» (Illumina, USA) and Next Generation Sequencing (NGS) was performed using a «MiSeq reagent kit version 2» with 2 × 250-bp paired-end sequencing on a MiSeq benchtop sequencer (Illumina, USA). In order to assemble each of the genomes, reads were mapped to the reference genome (FR682468.1_ASFV/Georgia 2007/1) and a consensus sequence was generated for each of the isolates (CLC Genomics Workbench v.9.5.2 (QIAGEN, Aarhus; www.clcbio.com). The original set of reads were subsequently mapped to the newly assembled virus genome, with an average coverage depth of 45× and an average read length of 250 nucleotides (nt). Open reading frames (ORFs) were predicted using GATU software. The complete genome sequences of both high passage strains were deposited in GenBank with accession numbers MW528217 and MW528218.

The complete genome sequences of previously characterized ASFV isolate Odintosovo 02/14 (KP843857.1) and the genotype II reference, ASFV/Georgia 2007/1 (FR682468.1), were included in subsequent genetic analysis. The sequences of two reference and the two cell culture adapted strains were used to generate an alignment, which in turn was used to detect single nucleotide polymorphisms (SNPs). Both the construction of the alignment and SNPs detections were performed using CLC Genomics Workbench v.9.

Results

Since the biological and genetic characteristics of the ASFV isolate Odintsovo 02/14 has been studied, the isolate was used during progressive adaption of the virus to continuous cell culture using fibroblast-like pseudodiploid cells from the African green monkey (*Cercopithecus aethiops*) (CV-1) [6]. The complete genomes of cell culture adapted variants from passage 30 and 50 (referred to as: ASFV/ARRIAH/CV-1/30 and ASFV/ARRIAH/CV-1/50 respectively) were determined.

The complete genomes of the analyzed viruses differ in length with ASFV/ARRIAH/CV-1/30 and ASFV/ARRIAH/CV-1/50 consisting of 186,529 bp and 186,525 bp respectively, while in comparison the reference strain Georgia 2007/1 and parental isolate Odintsovo 02/14 was 189,344 bp and 189,122 bp respectively. This indicates a large deletion within the genomes of the highly passaged strains. Total 78 SNPs were identified between the parental Odintsovo 02/14 and the two high passaged strains. These could be further classified as 13 SNPs within the intergenic regions and 5 associated with homopolymer repeat sequences. The SNPs within open reading frames were characterized as 8 synonymous SNPs and 52 non-synonymous SNPs. All 60 synonymous and non-synonymous SNPs are listed in **Table 1**.

Predicted proteins with conservative non-synonymous single nucleotide polymorphisms and synonymous in light grey. The amino acid predicted for each isolate and strain is listed as well as its position. Amino acid exchanges marked with dark grey indicate positions where either ASFV/ARRIAH/CV-1/30 or Odintsovo 02/14 were affected.

The majority (n = 36) of the non-synonymous SNPs are conservative amino acid exchanges, while 14 resulted in an exchange of a charged amino acid with either an amino acid with a different or no charge. Two (n = 2) of the non-synonymous SNPs resulted in early termination of the predicted protein (**Table 1** and **Table 2**). The predicted proteins affected by the early termination as well as the non-conservative amino acid exchanges are listed in **Table 2**.

The presented results clearly demonstrate that the majority of amino acid exchanges occurred in the genome of the adapted ASFV/ARRIAH/CV-1/50 and/or ASFV/ARRIAH/CV-1/30 variants, with the exception of the mutations highlghted in gray, which were unique to the genome of isolate Odintsovo 02/14.

Discussion

Under certain conditions, ASFV, a double-stranded DNA virus, may not have a high level of genome variability, but since no effective vaccine have been developed till the moment against African swine fever, it is of high importance to understand and study the relation between certain biological properties and changes in the virus genome.

Since it was previously described how adaptation of the ASFV to growth in a continuous cell culture leads to significant changes in its genome and a decrease in virulence [3, 7], complete genome sequencing and analysis is the most informative approach to understand the effect of these mutations on the pathogenesis of the virus.

In the presented study, it is clearly demonstrated that adaptation of the ASFV to growth in CV-1 continuous cell culture leads to the appearance of a large deletion in the 3’ variable region of the genome. Comparative analysis of the genome of the original isolate and the adapted variants also revealed 78 oligonucleotide polymorphisms that influenced or did not affect the predicted amino acid sequence of the encoded protein.

Only 2 oligonucleotide substitutions in the MGF_110_1L and B354L genes led to the formation of stop codons, which makes it possible to exclude the possibility of a complete synthesis of proteins encoded by these genes when pigs are infected with ASFV/ARRIAH/CV-1/50 strain.

Conclusion

The most significant modification in the genomes of ASFV/ARRIAH/CV-1/30 and ASFV/ARRIAH/CV-1/50 was a large-size (2947 bp) deletion in the right variable region of adapted viruses, between 181,980–184,929 bp. A similar deletion was described in the genome of another cell culture adapted strain, BA71V, where the same seven predicted proteins were affected (**Table 2**) [2].

The importance of each of the described mutations on cell adaptation, virus growth and virulence requires additional investigations.

Table 1. Genes of different strains of the African swine fever virus encoding proteins with synonymous and non-synonymous single nucleotide polymorphisms

Protein encoding gene	Virus strain				Predicted amino acid substitutions in the protein*
	Georgia 2007/1	Odintsovo 02/14	ASFV/ARRIAH/CV-1/30	ASFV/ARRIAH/CV-1/50	
ASFV_G_ACD_00270	R	R	R	K	R16K
ASFV_G_ACD_00270	R	R	R	K	R20K
MGF-360-11L	F	F	F	F	F120F
MGF-360-14L	L	L	L	F	L118F
A104R	T	T	T	I	T22I
MGF-360-15R	R	R	R	K	R60K
A859L	S	S	S	F	S244F
A859L	Y	Y	Y	F	Y614F
A859L	F	F	F	F	F684F
F317L	S	S	S	F	S206F
F77R	Q	Q	Q	Q	Q65Q
F1055L	I	I	I	F	I596F
ASFV_G_ACD_00760	L	L	L	I	L24I
K205R	S	S	S	S	S192S
EP1242L	Y	Y	Y	F	Y817F
EP1242L	S	S	S	F	S998F
EP1242L	L	L	L	F	L1145F
EP84R	L	L	L	F	L37F
EP153R	L	L	L	F	L10F
EP364R	C	C	C	G	C158G
M1249L	P	P	L	P	P1055L
M1249L	P	P	S	P	P1093S
M1249L	P	P	L	P	P1248L
C257L	L	L	L	F	L176F
C962R	T	T	T	N	T643N
C962R	L	L	L	L	L645L
B962L	L	L	L	F	L228F
B318L	I	I	I	F	I170F
B318L	L	L	L	F	L294F
B354L	I	I	I	I	I101I
B125R	D	D	D	E	D27E
G1340L	D	D	D	E	D979E
0174L	S	S	S	F	S75F
0174L	S	S	S	F	S121F
NP1450L	L	L	L	F	L446F
NP419L	N	N	N	S	N414S
H359L	A	A	A	T	A225T
H359L	I	I	I	F	I247F
E301R	D	D	D	E	D162E
E199L	F	S	F	F	F133S
I267L	S	S	S	F	S65F
ASFV_G_ACD_01990	F	F	F	F	F48F
DP60R	F	F	F	F	F27F
DP60R	V	V	V	F	V28F

Note. The amino acid predicted for each strain and its position are listed in the column «Predicted amino acid substitutions in the protein»; the positions affected in ASFV/ARRIAH/CV-1/30 or Odintsovo 02/14 strains are also listed there. Synonymous polymorphisms are highlighted in light gray; nucleotide changes that led to amino acid substitutions are marked in dark gray;

* – the first letter represents the amino acid of the wild type gene (the numbers indicate the position in the amino acid sequence of the protein), and the second letter represents the amino acid after the mutation.

Table 2. List of predicted amino acid substitutions with either charge changes or generation of stop-codon

Protein encoding gene	Virus strain				Predicted amino acid substitutions in the protein*
	Georgia 2007/1	Odintsovo 02/14	ASFV/ARRIAH/CV-1/30	ASFV/ARRIAH/CV-1/50	
MGF_110_1L	W	W	W	Stop-codon	Stop codon at position 197
MGF_360_8L	E	E	E	K	E70K
MGF-505-7R	D	D	D	N	D204N
MGF_505_6R	E	E	E	K	E378K
MGF_505_6R	E	E	E	K	E475K
F334L	M	M	M	K	M145K
F1055L	D	D	D	N	D576N
F1055L	E	E	E	K	E575K
EP1242L	D	D	D	N	D700N
C717R	D	D	D	N	D313N
B318L	Q	Q	Q	K	Q277K
B354L	E	E	E	Stop-codon	Stop codon at position 218
NP419L	D	D	D	N	D322N
E199L	G	E	G	G	G127E
E24R	D	D	D	N	D86N
DP96R	D	G	D	D	D64G
I7L	+	+			
I8L	+	+			
ASFV_G_ACD_001870	+	+			
I9L	+	+	2947 bp deletion (979 amino acids)	2947 bp deletion (979 amino acids)	No protein
I10L	+	+			
I11L	+	+			
MGF_360-18R	+	+			

Note. Mutations unique to the genome of the Odintsovo 02/14 strain are marked in gray;

* – the first letter represents the amino acid of the wild type gene (the numbers indicate the position in the amino acid sequence of the protein), and the second letter represents the amino acid after the mutation.

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