An immortalized porcine alveolar macrophage cell line with potential for live ASFV vaccine production

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Introduction

In addition to the development of suitable vaccine strains, the commercialization of live virus ASF vaccines requires cell lines capable of stable production.

Porcine alveolar macrophages (PAMs) and monocytes are commonly used for ASFV production in the laboratory. However, these primary cells have several disadvantages for use at industrial scale. ASFV-susceptible primate-derived cell lines (e.g., COS or Vero) provide an alternative, but the *in vivo* replication ability is often decreased in multi-passaged ASFV strains (1,2).

We have developed a PAM-derived cell line (iPAM) for the propagation of porcine reproductive and respiratory syndrome virus (PRRSV). Since the iPAM cell line combines the advantages of primary cells and established cell lines, we tested the susceptibility of iPAM to ASFV strains (genotype I and II). Our goal was to investigate the applicability of the cell line for ASFV production.

Results

iPAM cells were infected with one genotype I (NHV) and six genotype II (HU-2018, Georgia-2007/1 (G), Lv17 and gene-deleted derivatives) ASFV strains. With the Lv17 strains an order of magnitude higher virus titers were obtained in iPAM than in PAM (Figure 1). NHV grew with a half order of magnitude higher titer in PAM cells, while infection with the HU-2018 and G-Δ9GL/ΔUK/mut strains resulted in fairly similar titers in both cell types (Figure 1).

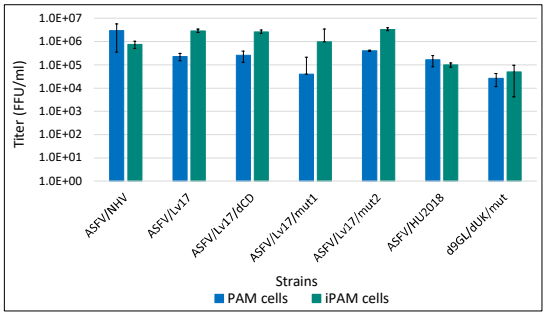

Figure 1. Infectious titer (FFU/ml) of seven ASFV strain in PAM (blue) and iPAM (green) cells at 72 h.p.i.

The genome stability of four different genotype II vaccine candidate strains (three Lv17/WB/Rie1 and one Georgia-2007/1 derivatives) were studied by NGS (Illumina platform) sequencing after 5 rounds of cell culture (iPAM) passage. Sequences were assembled from more than 10 million reads.

Two of the Lv17- and the Georgia-derived viruses did not show significant genetic changes after five passages (Table 1, 2 and 3). Genetic instability was only detected for Lv17/WB/Rie1/mut2 in the form of minor genome rearrangements (between nts 21939-22020 and 140488-140497) and single nucleotide polymorphisms (Table 4).

Table 1. Lv17/WB/Rie1-ΔCD pass5 compared to Lv17/WB/Rie1-ΔCD

Position	Change	Polymorphism Type	Variant Frequency	Change in protein	Affected ORF
20,009	C->Y	ambiguous	33%	A->T	MGF 300-2R
45,806	A->R	ambiguous	7%	D->G	MGF 505-10R
170,140	10A->11A	insertion	18.%	Frame shift	I226R
173,468	T->Y	ambiguous	7%	N->S	I177L

Table 2. Lv17/WB/Rie1-mut1 pass5 compared to Lv17/WB/Rie1-mut1

Position	Change	Polymorphism Type	Variant Frequency	Change in Protein	Affected ORF
4,067	G->R	ambiguous	9%	S->F	MGF 360-2L
40,029	T->W	ambiguous	5%	Y->N	MGF 505-10R
40,244	C->S	ambiguous	5%	D->E	MGF 505-10R

Table 3. G-Δ9GL/ΔUK/mut pass5 compared to G-Δ9GL/ΔUK/mut

Position	Change	Polymorphism Type	Variant Frequency	Change in Protein	Affected ORF
46,832	W (T->A)	ambiguous	9%	Y->N	MGF 505-10R

Table 4. Lv17/WB/Rie1-mut2 pass5 compared to Lv17/WB/Rie1-mut2

Position	Change	Polymorphism Type	Variant Frequency	Change in Protein	Affected ORF
3,034	C->S	quasispecies	19%	R->S	MGF360-2L
21,939-22,020	MTTMAACGTG GCTTMTTTMA AAGTTMCACA AYGTTMAAATC TCTACTTMCTT TMATTCTTTGT GGGGTTTTMT TAACTTTM	genetic instability, multiple variant	13%	NCR	NCR
60,066	T->K	ambiguous	13%	stop codon	F778R
60,192	C->M	ambiguous	23%	T->N	F778R
71,005	A->M	ambiguous	16%	stop codon	EP1242L
71,038	A->M	ambiguous	19%	V->G	EP1242L
71,524	A->M	ambiguous	11%	I->L	EP84R
71,528	A->M	ambiguous	22%	Y->S	EP84R
111,660	T->K	ambiguous	19%	Y->S	G1340L
140,488-140,497	CCGCGACATC->YCGYGRSRTY	genetic instability, multiple variant			D339L
152,204	T->K	ambiguous	22%	I->R	H339R
154,222	T->K	ambiguous	16%	I->R	H339R

The variant frequencies remained below 10% in the case of G-Δ9GL/ΔUK/mut and Lv17/WB/Rie1-mut1 (Table 2 and 3), while higher than 20% and 30% frequencies were detected in the case of Lv17/WB/Rie1-mut2 and Lv17/WB/Rie1-ΔCD respectively (Table 1 and 4).

Conclusions

- The iPAM cell line supports high-titer growth of genotype I and II strains.
- It maintains the genetic integrity of ASFV well.
- The cells have the potential to replace primary macrophages in many areas of ASFV research.
- Because iPAM cells can grow in suspension, virus production can be easily upscaled to bioreactors.
- Therefore, we believe that iPAM cells are suitable for large-scale vaccine production and allow regulatory approval of an ASF vaccine.

References

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