BMC Veterinary Research

RESEARCH Open Access

pUS6 in pseudorabies virus participates in the process of inhibiting antigen presentation by inhibiting the assembly of peptide loading complex

Ningning Ma[1,2], Yawei Sun[1,2], Chenmeng Ding[1,2], Yongtao Li[1,2], Linyang Yu[1,2*] and Lu Chen[1,2*]

Abstract

Pseudorabies virus (PRV) can establish lifelong latent infection in peripheral nervous ganglion, and persistent infections in peripheral blood lymphocytes. Establishing an infection in the lymphocytes does not only enable the PRV to escape host immune surveillance but pass through the placental barrier, leading to fetal death and abortion. Due to the pathogenicity of the PRV, it poses a huge challenge in its prevention and control. The PRV escapes host immunity through downregulation of swine leukocyte antigen class I (SLA I) molecules on infected cells. However, data on the molecular mechanisms of the SLA I suppression remains scant. Here, in order to verify the effect of candidate proteins PRV pUL44 and pUS6 on PRV immune escape related molecules SLA I and peptide loading complex (PLC), we detected the expression of SLA I and PLC components after expressing PRV pUL44 and pUS6. The effects of pUS6 and pUL44 on SLA I and PLC were analyzed by qRT-PCR and Western blot at mRNA and protein level, respectively. Cells expressing pUS6 or pUL44 genes showed a significantly suppressed expression of surface and total SLA I molecules. In addition, unlike UL44, the US6 gene was shown to downregulate the transporter associated with antigen processing 1 (TAP1), TAP2 and Tapasin molecules. The results show that PRV pUS6 may participate in virus immune escape by directly regulating the SLA I, TAP dimer and Tapasin molecules, thus blocking the transportation of TAP-bound peptides to the ER to bind SLA I molecules. We provide a theoretical basis on the mechanism of TAP mediated immune escape by the PRV.

Keywords Pseudorabies virus, Immune escape, SLA I, PLC, TAP, Tapasin

*Correspondence:
Linyang Yu
linyangyuhenau@163.com
Lu Chen
chenluhau@126.com
[1]College of Veterinary Medicine, Henan Agricultural University, 450046 Zhengzhou, People's Republic of China
[2]Ministry of Education Key Laboratory for Animal Pathogens and Biosafety, Henan Agricultural University, 450046 Zhengzhou, People's Republic of China

Introduction

Pseudorabies virus (PRV) is a contagious virus that causes pseudorabies (PR). Thevirus causes abortion in sows, sterility in breeding boars, as well as diarrhoea and neurological symptoms in newborn piglets, which has brought substantial global economic losses to the pig industry [1, 2]. As a member of the *Herpesviridae* family, PRV has devised strategies to escape host immune responses to establish a latent state, which leads to a persistent infection. Under stressful conditions, the latent PRV is reactivated, posing a huge challenge in the prevention and treatment of PRV [3]. Under normal circumstances, a proportion of viral proteins are rapidly degraded into peptides by the proteasome. Then, under the action of the peptide assembly complex, the peptides are assembled with MHC I. The complexes are assembled under the synergistic action of multiple proteins located in the endoplasmic reticulum (ER) [4, 5]. Transporter associated with antigen processing (TAP) is a heterodimer composed of TAP1 and TAP2 subunits that form part of the PLC. Besides, PLC contains several helper proteins, such as Tapasin, calcitonin (CNX), calreticulin (CRT), Endoplasmic reticulum protein (ERp) or β2m. Tapasin is an important bridge connecting TAP and the remaining components of the PLC, and plays a vital role in stabilizing the TAP structure and the entire PLC assembling process [6, 7]. During MHC I antigen presentation, Nascent MHC I is bound by the chaperone calreticulin. At this stage the oxidoreductase ERp57 and Tapasin are recruited to the loading complex. A relatively stable disulfide bond forms between Tapasin and ERp57, forming the core functional unit of the PLC. Tapasin colocalizes this peptide receptive loading complex to the TAP heterodimer, where peptides generated in the cytoplasm by the proteasome are translocated into the lumen of the ER. Following peptide loading, TAP translocates peptides across the ER membrane via a conformational transition that is energized by the hydrolysis of ATP, the class I molecules dissociate from the TAP and associated chaperones and are transported to the cell surface where they are scrutinized by CD8+T cells [8]. Abnormal expression of any gene involved in this pathway can directly affect the expression of MHC I molecular complex on the cell surface.

Many herpes viruses have evolved strategies to interfere with this process thereby evading the host immunity [5, 9, 10]. The strategies involve inhibiting proteasome degradation to produce antigenic peptides, inhibiting the function of TAP in presenting antigenic peptides, retaining the TAP in the ER cavity combined with MHC I, exporting the MHC I out of the ER, transferring newly assembled chains into lysosome for degradation, as well as promoting cell surface MHC I endocytosis by the lysosomes [5]. In addition, deletion of Tapasin may lead to inadequate intracellular assembly which might affect the expression of MHC I molecules [11]. Previous studies have demonstrated that the membrane protein pUL49.5, encoded by varicella virus, bovine herpesvirus type 1 (BHV-1), equine herpesvirus type 1 and 4 (EHV-1 and EHV-4), not only inhibits the conformational rearrangement of peptide binding to ATP but also significantly downregulates the expression of TAP1 or TAP2 proteins by inducing proteasome-specific degradation of TAP1 and TAP2 subunits, thereby interfering with the assembly and transport of MHC I (Swine leukocyte antigen class I, SLA-I). This sequence of events facilitates the evasion of viral particles from the host's innate immune response. ICP47 proteins of herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2) are soluble cytosolic proteins acting as high-affinity competitors for peptide binding to the TAP [12, 13]. The US6 protein encoded by human cytomegalovirus (HCMV) inhibits TAP function by reducing the interaction between ATP and TAP [14–16]. Besides, the BNLF2a protein in the Epstein-Barr virus (EBV) and primate gamma-1 herpesviruses is considered to be an effective TAP inhibitor, which can prevent the binding of peptides and ATP to TAP [17, 18]. These proteins appear to be remarkably heterogeneous in function. In addition, PRV variants down-regulates SLA I molecules on the surface of PK-15 cells and targets them for lysosomal degradation [5, 19].

Previous studies have confirmed that PRV pUL49.5 inhibits the function of TAP to transport antigenic peptides by blocking the basic conformational changes of TAP. Besides, there is limited data on the molecular mechanisms of inhibiting the function of TAP. In the previous research, we preliminarily screened pseudorabies virus proteins affecting TAP peptide transport by bioinformatics. The hydrophobic proteins PRV pUS6 and pUL44, which possess a signal peptide and a transmembrane domain, predominantly localize to the plasma membrane and ER. These proteins exhibit an identical structural composition as HCMV US6, as confirmed through screening. Therefore, we chose US6 and UL44 as the research object [20]. In this study, transfection of pUS6-HA and pUL44-HA into PK-15 cells was performed to investigate the impact of candidate proteins on the transcription or translation of SLA I and PLC components. The verification was conducted using qRT-PCR and Western blot techniques, aiming to establish a theoretical foundation and gain insights into the immune evasion mechanisms employed by PRV. Additionally, this research aims to identify novel antiviral targets and provide a scientific basis for the development of new vaccines.

Materials and methods

Primer design

We used Primer 5.0 software to design specific primers (PRV UL44-F/R and US6-F/R) for the PRV HN1201 sequence (accession number: KP722022.1). The PRV UL44-F/R or US6-F/R primers were used to amplify full length PRV US6 or UL44 genes, respectively (Table 1). The restriction sites, *Kpn I* and *Xho I*, as well as the homologous sequence of 15 bp pCAGGS-HA vector were added to the UL44-F/R 5' terminal for recombination. Additionally, *EcoRI* and *XhoI* restriction enzyme recognition sites were strategically incorporated at the 5' terminus of US6-F/R for recombination, along with a 15 bp homologous sequence derived from the pcDNA3.1 (+)-HA vector. The specific primers UL44-qF/R or US6-qF/R were used to amplify the mRNA of the PRV UL44 or US6 respectively. In addition, to amplify the TAP2 mRNA, we designed the TAP2 primers (TAP2-qF/R) based on the sequence published by NCBI GenBank (NM_001206441.1). In contrast, for the amplification of SLA I, TAP1, Tapasin, ERp, CRT, or CNX mRNA, we employed previously described primers [21]. The above primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd(Table 1).

Table 1 Primer sequence information

Primer name	Sequence (5′→3′)	Gene	bp
PRV UL44-F	TCGAGCTCATCGA***TGGTA***CCATGGCCTC GCTCGCGCGTGCG	PRV UL44	1464
PRV UL44-R	ATTAAGATCTGCTAG***CTCGAG***CAGCGCG GACCGGCGGTAGTA		
PRV US6-F	TAGTCCAGTGTGGTG***GAATTC***ATGCTGC TCGCAGCGCTATTG	PRV US6	1209
PRV US6-R	AACGGGCCCTCTAGA***CTCGAG***CAGCGC GGACCGGCGGTAGTA		
PRV UL44-qF	GGCGCAAGCGCATCGTGT	PRV UL44	139
PRV UL44-qR	GCGGGGGCTCGTCAAAGT		
PRV US6-qF	GCAAGAGTGCCCGTTCGCC	PRV US6	42
PRV US6-qR	GCCCCGCTTGAAGCTGTCGT		
SLA I-qF	AAGTCAAGGAAACCGCACAG	SLA I	113
SLA I-qR	CAAGTAGCAGCCAAACA TGC		
TAP1-qF	TGGGCTCCTCAAAGGAAA TA	TAP1	119
TAP1-qR	GGCAAAGGAGACATTCTGGA		
TAP2-qF	GCTGTTCTCGGGGTCTGTGA	TAP2	124
CRT -qF	AGCGAGCCAAAA TTGA TGAC		124
CRT-qR	TCCCACTCTCCA TCCA TCTC	CRT	
CNX-qF	GCAGAGAAGCCAGAGGA TTG	CNX	116
CNX-qR	ATCATAGGTCGCTGCCAGAC		
Tapasin-qF	TACACCTGCCATACCTGCAA	Tapasin	137
Tapasin-qR	AGACACCAGGCAGAGCAACT		
ERp-qF	TGTTTGCCAGAGTTGA TTGC	ERp	125
ERp-qR	TCGCTGACCCCTGTA TTCTC		
β-actin qF	TCCTGCGGCATCCACGAAAC	β-actin	82
β-actin qR	CCGTGTTGGCGTAGAGGTCCTTG	β-actin	

Construction and identification of plasmids expressing pUL44 or pUS6

PRV UL44 and US6 were amplified using the UL44-F/R and US6-F/R primers, respectively. The resultant 1464 bp UL44 product was ligated into the *Kpn I/XhoI* sites of a eukaryotic vector pcDNA3.1(+)-HA to obtain the pUL44-HA plasmid. Besides, the resulting 1206 bp US6 product was ligated into the *EcoR I/XhoI* sites of the eukaryotic vector pcDNA3.1(+)-HA to obtain the pUS6-HA plasmid. These plasmid constructs were then validated using restriction digestion and sequencing (Huada Gene, China).

Expression of PRV US6 and UL44 in PK-15 cells

We evaluated the pUS6-HA and pUL44-HA mRNA expression by quantitative qRT-PCR as described previously [20]. Briefly, PK-15 cells were seeded on 6-well plates (Greiner Bio-One) and incubated overnight before being transfected with 3 μg of pUS6-HA or pUL44-HA. Cell lysates were harvested using the RNA prep Pure Cell/Bacteria Kit (TIANGEN BIOTECH BEIJING CO., LTD) at 6, 12, 18 and 24 h post-transfection following the manufacturer's protocol. After reverse transcription, we performed qRT-PCR with UL44-qF/R or US6-qF/R primers (Table 1), while β-actin gene served as an internal control. Western blot assay was performed as described previously to profile the PRV pUS6 and pUL44 protein expression in the PK-15 cells [5]. The eukaryotic expression plasmid was transfected into the PK-15 cells in a 6-well plate. After 30 h, the cells in PBS were chilled on ice and detached with a cell scraper. The cells were span at 300 g for 5 min, and then the pellets lysed in 150μL RIPA buffer with a protease inhibitor cocktail. The extracted total proteins were quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific Pierce, USA). The total proteins were lysed in 2× Laemmli sample buffer containing 5% β-mercaptoethanol and then resolved in an 1%SDS-PAGE. The proteins were transferred into polyvinylidene fluoride (PVDF) membranes and blocked with 5% skimmed milk in 1×PBS. Thereafter, the membranes were incubated with respective primary antibodies for 1.5 h (Anti-hemagglutinin (HA) monoclonal antibodies (Mab), anti-β actin Mab were purchased from Sigma-Aldrich (St. Louis, MO, USA)., followed by horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h, and then subjected to enhanced chemiluminescence (ECL, Pierce).

Effect of PRV pUL44 or pUS6 on SLA I molecules or the components of peptide loading complex

The total RNA was extracted for reverse transcription at 6, 12, 18, and 24 h post-transfection with 3 μg of pUS6-HA or UL44-HA. Subsequently, the impact of the candidate proteins on SLA I and PLC mRNA components was assessed using quantitative real-time PCR (qRT-PCR). ChamQ SYBR qPCR Master Mix (Without ROX) and HiScript II Q RT SuperMix for qPCR were procured from Vazyme Biotech Co., Ltd. In addition, to verify the expression of SLA I molecules on the cell membrane surface, the cells membrane proteins from the different experimental groups were extracted according to the Sangon Biotech membrane protein extraction kit (Sangon Biotech (Shanghai) Co., Ltd.). Conversely, for confirming the expression of total SLA I or TAP1 protein, total proteins were extracted as previously described and verified by Western blot analysis [5].

Statistical analysis

The data was analyzed using GraphPad Prism software (version 8.0). All measurements between different groups were analyzed by t-test. The data are expressed as mean ± standard deviation. The error bar represents the standard deviation from the mean value of 3 repetitions, and the value of $P < 0.05$ is considered to be statistically significant. The Western blot data was analyzed by ImageJ software, and the expression was normalized by dividing the gray value of the target protein with the gray value of the internal reference protein.

Results

Expression of PRV pUS6 and pUL44 in the transfected cells

Using in silico tools, we screened the PRV pUS6 and pUL44 with similar structure to TAP inhibitor HCMV US6 from PRV encoded proteins [20] The pUS6-HA and pUL44-HA eukaryotic expression plasmids were constructed by homologous recombination for functional characterization of candidate proteins. To determine the expression of pUS6 and pUL44 in PK-15 cells, the mRNA and protein expression levels of the genes were analyzed by qRT-PCR and Western blot, respectively. Our data demonstrated a significant upregulation of US6 mRNA expression at 18 h post-transfection, with further enhancement observed at 24 h (Fig. 1A). Besides, there was a notable increase in UL44 mRNA expression specifically at 24 h post-transfection (Fig. 1B). However, compared to US6, the expression level of UL44 mRNA was relatively lower, potentially indicating gene-specific or vector-related disparities. Western blot analysis also confirmed the presence of PRV US6 and UL44 proteins in PK-15 cells (Fig. 1C). Thus, both pUL44-HA and pUS6-HA recombinant plasmids exhibited successful expression in PK-15 cells.

PRV pUS6 or pUL44 downregulates the SLA I expression

To determine whether the pUS6 or pUL44 protein mediates virus immune escape, the eukaryotic expression plasmids were transfected into the PK-15 cells, and PRV WT infected cells were used as a positive control. The SLA I mRNA and protein expression was analysed by qRT-PCR and western blot, respectively. Compared with the control group, transfection with PRV pUS6 or pUL44 resulted in significant suppression of the SLA I mRNA expression in PK-15 cells at 6 h, which decreased further at 24 h (Fig. 2A, B). Moreover, transfection with PRV pUS6 and pUL44 led to significantly reduced expression of cell surface content and total SLA I protein. Furthermore, data from the effects of the SLA I in the PRV-infected PK-15 cells showed that the inhibition efficiency on SLA I expressions was higher in infected PK-15 cells than in transfected PK-15 cells (Fig. 2C). These results indicated that pUS6 inhibited SLA1 expression.

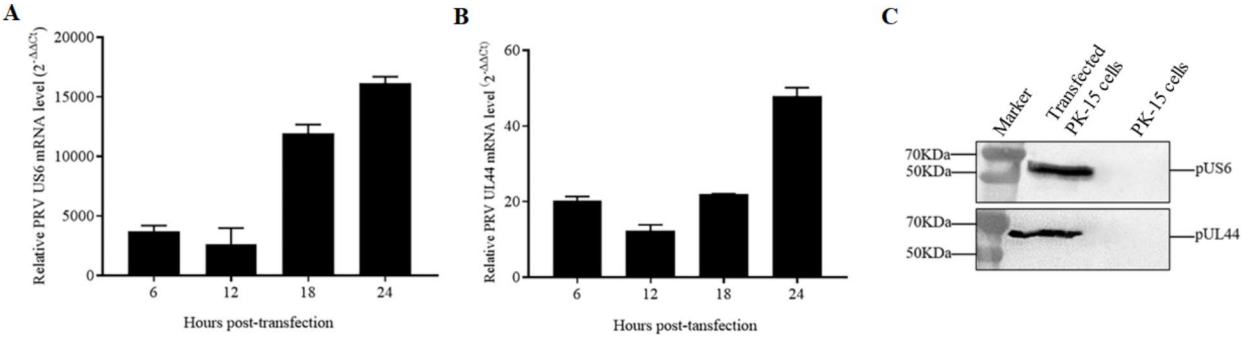

Fig. 1 Expression of PRV pUS6 or pUL44 in PK-15 cells. (**A**, **B**) PK-15 cells were transfected with 3 μg of pHA-US6 or pHA-UL44 and harvested at 6, 12, 18 and 24 h post-transfection, while the cells used for protein detection were collected 30 h post-transfection. The mRNA profile of PRV US6(A) or UL44(B) was assayed by qRT-PCR. (**C**) The pUS6 or pUL44 protein expression was measured by Western blot. The data are presented as mean ± standard deviation, from three independent experiments. Statistically significant values are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

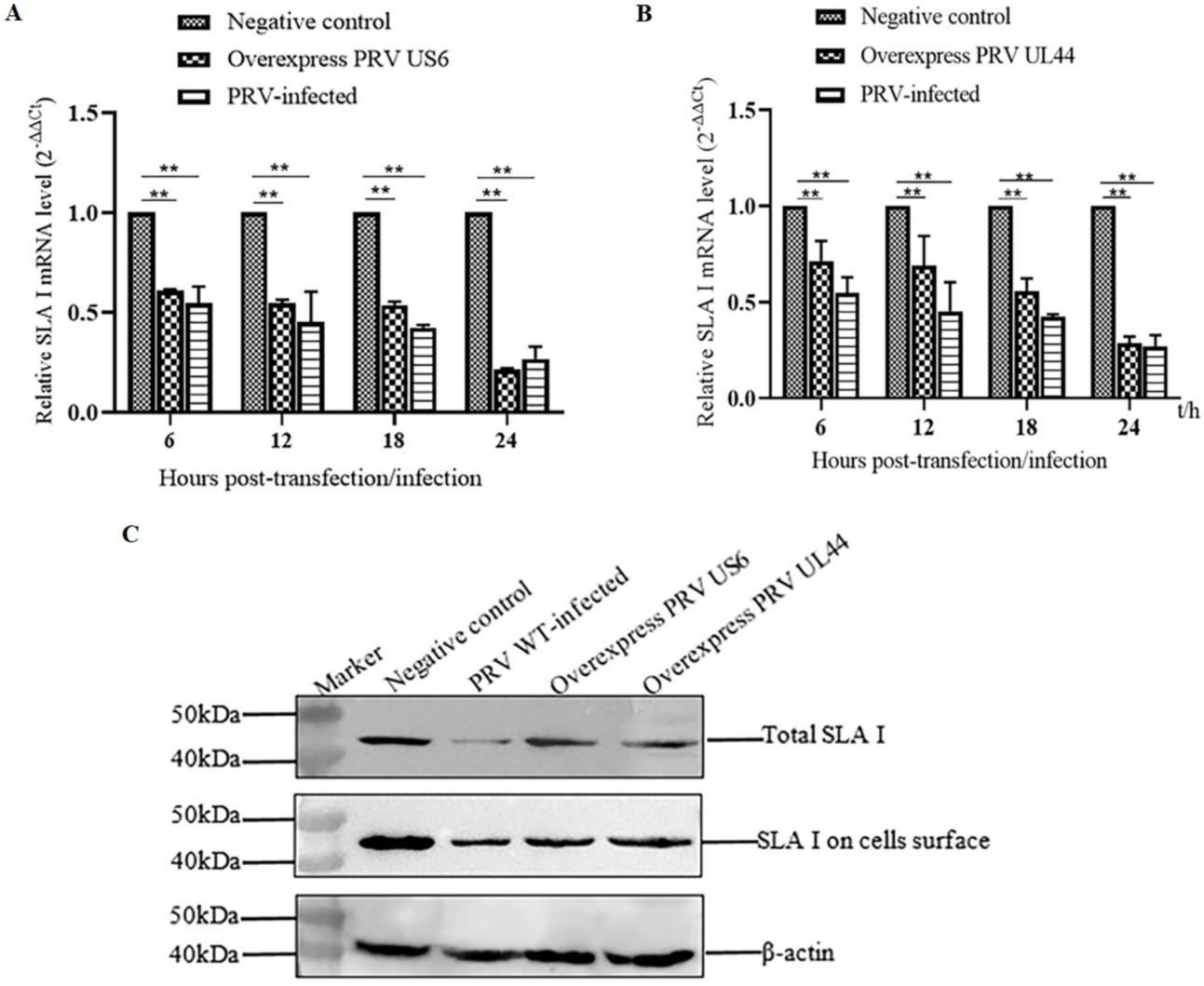

Fig. 2 PRV pUS6 and pUL44 down-regulate total and cell surface SLA I molecules. PK-15 cells were transfected with 3 μg of pUS6-HA or pUL44-HA or infected with PRV at an MOI of 1. The cells were harvested at 6, 12, 18 and 24 h post-transfection or infection, while the cells used for protein detection were collected 30 h post-transfection. (**A**, **B**) The mRNA profile of SLA I was measured by qRT-PCR method. (**C**) The protein levels of total and cell surface expression of SLA I molecules were measured by Western blot. (D, E) The optical density ratios of SLA I/β-actin were analysed using ImageJ. For the detection of cell membrane surface protein samples, a specific cell membrane surface protein extraction kit is used. The data are presented as mean ± standard deviation from three independent experiments. Statistically significant values are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

The down-regulation of SLA I molecules by PRV pUS6 is associated with suppression of TAP expression

TAP, a channel for peptides to enter the ER from the cytoplasm, plays a pivotal role in the entire antigen presentation process [21]. Some viruses inhibit the function of TAP by blocking the conformational changes necessary for peptide transport, and by inducing the degradation of TAP complex. To investigate whether the observed down-regulation of surface SLA I is contingent upon TAP inhibition, PK-15 cells were transfected with pUS6-HA or UL44-HA, or infected with PRV. Total RNA was extracted, and the transcript levels of the TAP1 and TAP2 genes were assessed by qRT-PCR. The data showed that PRV US6 significantly inhibits the TAP1 or TAP2 mRNA at 18 h and 24 h post-transfection, respectively (Fig. 3A, B). Interestingly, PRV UL44 failed to down-regulate the TAP1 or TAP2 mRNA levels (Fig. 3C, D). Additionally, the cells were harvested post-transfection with pUS6 and subsequently subjected to western blot analysis. Notably, this analysis revealed a significant downregulation of TAP1 protein expression. Importantly, regardless of mRNA or protein levels, the inhibition of TAP in the PRV infection group was more pronounced compared to that observed in the PRV pUS6 transfection group. (Fig. 3E, F). In the absence of a functional TAP transporter, most SLA I molecules are not loaded with peptides. They are retained within the ER and ultimately directed for proteasomal degradation.

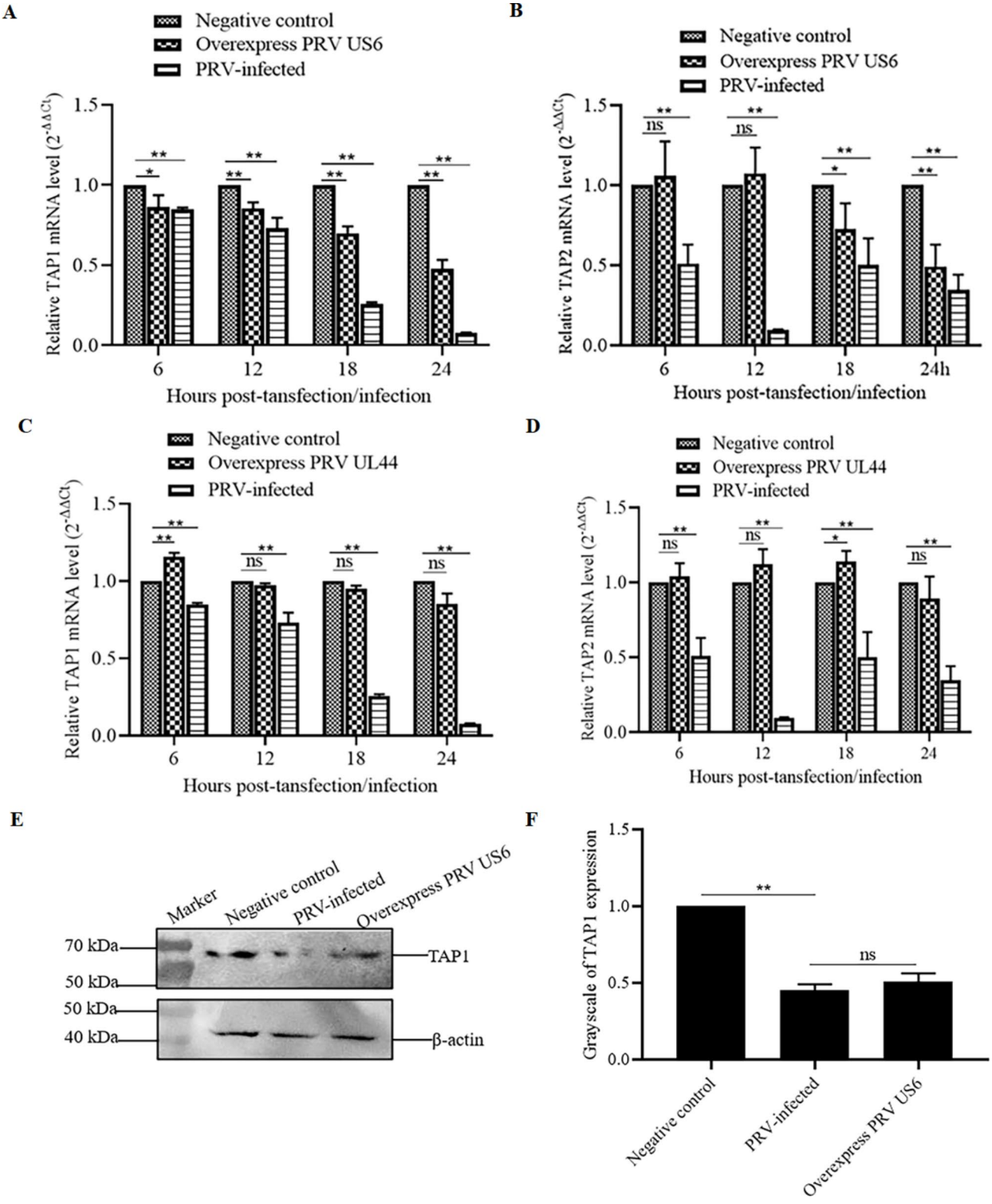

Fig. 3 PRV US6 down-regulates TAP1 or TAP2 molecules in cells. PK-15 cells were transfected with 3 μg of pUS6-HA or pU L44-HA, or infected with PRV at an MOI of 1. The cells were then harvested at 6, 12, 18 and 24 h post-transfection or infection and the mRNA levels of TAP1 or TAP2 were evaluated by qRT-PCR. (**A**, **B**) The effect of PRV US6 on intracellular TAP1 or TAP2. (**C**, **D**) The effect of PRV UL44 on intracellular TAP1 or TAP2. (**E**) The cells were harvested at 30 h post-transfection or infection by US6 followed by western blot analysis of TAP1 protein, with β-actin as an internal control. (**F**) The optical density ratios of TAP1/β-actin were analysed using ImageJ. The data are presented as mean ± standard deviation from three independent experiments. Statistically significant values are indicated by $^{*}p < 0.05$; $^{**}p < 0.01$

The down-regulation of SLA I molecule by PRV pUS6 is associated with the suppression of Tapasin

In addition to TAP, the PLC also includes Tapasin, CNX, CRT and ERp. These molecules synergize SLA I in their vital roles in cellular immuninty [21]. To interrogate whether the downregulation of SLA I on the cell surface is related to other components of the PLC, PK-15 cells were transfected with pUS6-HA or pUL44-HA. The total RNA was extracted, and the transcript levels of the CRT, CNX, ERp, or Tapasin genes were assessed using qRT-PCR. Whereas the data showed that the expression of PRV pUS6 significantly inhibited the mRNA levels of Tapasin genes at 6 h post-transfection, there were no significant changes in the mRNA levels of the CRT, CNX or ERp genes (Fig. 4A, B, C and D). Interestingly, PRV pUL44 did not down-regulate the mRNA levels of each component of thePLC. Further results suggest that Tapasin may be involved in the process of PRV pUS6 evading immune response.

Discussion

MHC I molecular antigen presentation pathway plays a central role in innate immunity. In the ER membrane, a dynamic multisubunit PLC is formed, providing an optimal platform for efficient MHC I assembly and peptide loading. Amongst these, the peptide transporter TAP is a very prominent target and antigen translocation into the ER is affected at any step in the transport cycle. Previous studies have shown that the loss of TAP1 or TAP2 protein function confer serious hinderance to the antigen presentation process of the MHC I molecules [22]. Tapasin bridges the TAP with the MHC I, providing a

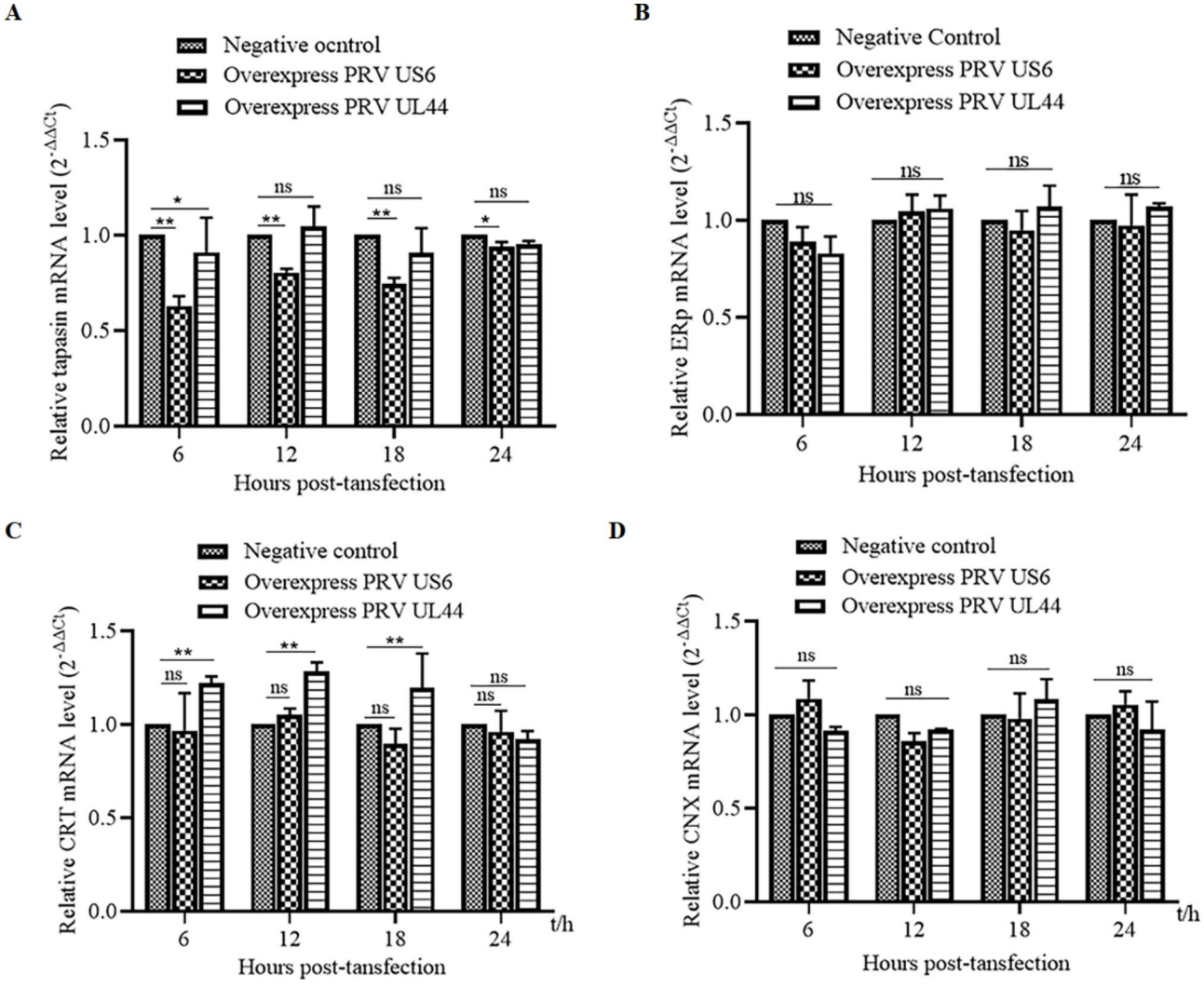

Fig. 4 The down-regulation of Tapasin molecule by PRV pUS6. PK-15 cells were transfected with 3 μg of pUS6-HA or pUL44-HA or infected with PRV at an MOI of 1. (**A**, **B**, **C** and **D**) The cells were harvested at 6, 12, 18 and 24 h post- transfection or infection and the mRNA levels of Tapasin, ERp, CRT and CNX were evaluated using qRT-PCR. The data are presented as mean ± standard deviation from three independent experiments. Statistically significant values are indicated by $*p < 0.05$; $**p < 0.01$

close proximity for optimal peptide loading. Notably, the tapasin-TAP interaction enhances the stability of the TAP complex, and Tapasin can effectively improve the expression of TAP and the transport efficiency by transmitting complex information changes [11]. In addition, two accessory chaperones, ERp and CRT, complete the PLC [23]. *Herpesviruses* independently developed distinct methods to block TAP. Using in silico tools, we screened the PRV pUS6 and pUL44 with similar structure to TAP inhibitor HCMV US6 from PRV encoded proteins [20]. To determine the expression of pUS6 and pUL44 in PK-15 cells, the mRNA and protein expression levels of the genes were analyzed by qRT-PCR and Western blot, respectively. Our data showed that the mRNA level of PRV US6 or PRV UL44 increased gradually from 12 h, but the overall expression level of UL44 was lower than that of US6. Western blot results also showed the expression of PRV US6 and UL44 in PK-15 cells, which might be gene-specific of vector-related differences. Evaluation of the expression levels of SLA I and TAP in PRV pUS6 or pUL44 transfected or PRV-infected cells showed significant decline in the expression of SLA I protein on the surface of PK-15 cells, mRNA and protein expression level of the total SLA I molecules, while only PRV pUS6 products significantly inhibited the expression of TAP1 and TAP2 genes. This suggests that PRV pUS6 downregulated the SLA I and TAP expression through either mRNA degradation or translation repression and inhibited the transport of antigens from the cytoplasm to the ER. In the absence of a functional TAP transporter, most SLA I molecules are not loaded with peptides. They are retained within the ER and ultimately directed for proteasomal degradation, thus aiding in the evasion of the host immune response. So, our data demonstrated that the expression of pUS6 and pUL44 can induce immune evasion by suppressing self-expression of SLA I molecules, impeding proteasome degradation for antigenic peptide production, inhibiting TAP-mediated presentation of antigenic peptides, facilitating lysosomal degradation of newly assembled chains, and promoting endocytosis of cell surface SLA I molecules into lysosomes for degradation. However, the mechanism by which PRV UL44 down-regulates cell surface SLA I molecules remains unclear. UL44 may exert inhibitory effects on PLC-promoting peptides and interfere with SLA I molecular assembly. Additionally, it could facilitate the export of SLA I from the endoplasmic reticulum (ER), promote transfer of newly assembled SLA I to lysosomes for degradation, and enhance endocytosis-mediated lysosomal degradation of cell surface SLA I molecules. These hypotheses require further experimental validation.

Finally, in addition to the TAP, we also studied whether the downregulation of SLA I by PRV pUS6 or pUL44 was also related with other components of the PLC, including Tapasin, CNX, CRT and ERp. The results showed that whereas the expression of PRV pUS6 significantly inhibited the Tapasin mRNA, the PRV pUL44 had no effect on the mRNA of each component of PLC. Tapasin is an important bridge between the TAP and other components of the PLC, it stabilizes the TAP complex and optimizes the binding peptide. Thus, the PRV pUS6 may also inhibit the assembly of peptide-SLA I complex by reducing the expression of Tapasin, thus down-regulating the SLA I molecule on the cell surface. Similar to previous reports where MRC-5 and HEK293 cells were used to study the surface of human cytomegalovirus antigen presenting component (APM), it was shown that there was suppression of TAP1, TAP2 or Tapasin gene transcription levels [24]. However, the mechanism with which the PRV pUL44 down-regulates the cell SLA I molecules remains unknown. We speculate that it might affect the immune response by inhibiting proteasome degradation to produce antigenic peptide, export of the SLA I out of the ER, transfer of newly assembled SLA I into the lysosome for degradation, or promotion of cell surface SLA I molecule endocytosis into lysosome.

Conclusions

Taken together, our data demonstrates that PRV pUS6 down-regulates the expression of SLA I, TAP dimer and Tapasin molecules. Conversely, PRV pUL44 exclusively attenuates SLA I expression. PRV pUS6 plays an important role in the process of virus immune escape by directly regulating the SLA I gene, TAP dimer and Tapasin molecules, thus blocking the transportation of TAP-bound peptides to the ER to bind SLA I molecules. Our findings do not only highlight the functions of PRV-encoded protein in viral immune escape, but provide new insights into the interactions between viral infections and their hosts.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04294-3.

Supplementary Material 1

Author contributions

Ningning Ma: Conduct experiments and data analysis, software, Writing – Review & Editing. Ningning Ma, Yawei Sun, Chenmeng Ding: Data collation of Western Blot. Ningning Ma, Yawei Sun, and Yongtao Li: Design primers. Linyang Yu, Lu Chen: Methodology, Review and revise the draft.

Funding

This work was supported by the National Natural Science Foundation of China (no. 31772781), The Key Science Foundation of Henan (no. 232300421108), The Postdoctoral Research Start-up Foundation of Henan (no. HN2022124).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval
Not applicable.

Consent to participate
Not applicable.

Consent to Publish
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 21 March 2024 / Accepted: 20 September 2024
Published online: 09 October 2024

References

1. Huang J, Zhu L, Zhao J, Yin X, Feng Y. Genetic evolution analysis of novel recombinant pseudorabies virus strain in Sichuan. China. 2020;67(4):1428–32. https://doi.org/10.1111/tbed.13484.
2. Yu X, Zhou Z, Hu D, Zhang Q, Han T, Li X, Tian K. Pathogenic pseudorabies virus, China, 2012. Emerg Infect Dis. 2014;20(1):102–4. https://doi.org/10.3201/eid2001.130531.
3. Hu RM, Zhou Q, Song WB, Sun EC, Zhang MM, He QG, Liu ZF. Novel pseudorabies virus variant with defects in TK, gE and gI protects growing pigs against lethal challenge. Vaccine. 2015;33(43):5733–40. https://doi.org/10.1016/j.vaccine.2015.09.066.
4. Bouvier M. Accessory proteins and the assembly of human class I MHC molecules: a molecular and structural perspective. Mol Immunol. 2003;39(12):697–706. https://doi.org/10.1016/s0161-5890(02)00261-4.
5. Zhao H, Wang S, Liu C, Han J, Tang J, Zhou L, Yang H. The pUL56 of pseudorabies virus variant induces downregulation of swine leukocyte antigen class I molecules through the lysosome pathway. Virus Res. 2018;251:56–67. https://doi.org/10.1016/j.virusres.2018.04.007.
6. Garbi N, Tiwari N, Momburg F, Hämmerling GJ. A major role for tapasin as a stabilizer of the TAP peptide transporter and consequences for MHC class I expression. Eur J Immunol. 2003;33(1):264–73. https://doi.org/10.1002/immu.200390029.
7. Leonhardt RM, Keusekotten K, Bekpen C, Knittler MR. Critical role for the tapasin-docking site of TAP2 in the functional integrity of the MHC class I-peptide-loading complex. J Immunol. 2005;175(8):5104–14. https://doi.org/10.4049/jimmunol.175.8.5104.
8. Purcell AW, Elliott T. Molecular machinations of the MHC-I peptide loading complex. Curr Opin Immunol. 2008;20(1):75–81. https://doi.org/10.1016/j.coi.2007.12.005.
9. Lu YC, Weng WC, Lee H. Functional roles of calreticulin in cancer biology. Biomed Res Int. 2015;2015:526524. https://doi.org/10.1155/2015/526524.
10. Wright CA, Kozik P, Zacharias M, Springer S. Tapasin and other chaperones: models of the MHC class I loading complex. Biol Chem. 2004;385(9):763–78. https://doi.org/10.1515/bc.2004.100.
11. Li S, Paulsson KM, Chen S, Sjögren HO, Wang P. Tapasin is required for efficient peptide binding to transporter associated with antigen processing. J Biol Chem. 2000;275(3):1581–6. https://doi.org/10.1074/jbc.275.3.1581.
12. Halenius A, Momburg F, Reinhard H, Bauer D, Lobigs M, Hengel H. Physical and functional interactions of the cytomegalovirus US6 glycoprotein with the transporter associated with antigen processing. J Biol Chem. 2006;281(9):5383–90. https://doi.org/10.1074/jbc.M510223200.
13. Herbring V, Bäucker A, Trowitzsch S, Tampé R. A dual inhibition mechanism of herpesviral ICP47 arresting a conformationally thermostable TAP complex. Sci Rep. 2016;6:36907. https://doi.org/10.1038/srep36907.
14. Hislop AD, Ressing ME, van Leeuwen D, Pudney VA, Horst D, Koppers-Lalic D, Wiertz EJ. A CD8+T cell immune evasion protein specific to Epstein-Barr virus and its close relatives in Old World primates. J Exp Med. 2007;204(8):1863–73. https://doi.org/10.1084/jem.20070256.
15. Kyritsis C, Gorbulev S, Hutschenreiter S, Pawlitschko K, Abele R, Tampé R. Molecular mechanism and structural aspects of transporter associated with antigen processing inhibition by the cytomegalovirus protein US6. J Biol Chem. 2001;276(51):48031–9. https://doi.org/10.1074/jbc.M108528200.
16. Praest P, Luteijn RD, Brak-Boer IGJ, Lanfermeijer J, Hoelen H, Ijgosse L, Wiertz E. The influence of TAP1 and TAP2 gene polymorphisms on TAP function and its inhibition by viral immune evasion proteins. Mol Immunol. 2018;101:55–64. https://doi.org/10.1016/j.molimm.2018.05.025.
17. Horst D, van Leeuwen D, Croft NP, Garstka MA, Hislop AD, Kremmer E, Ressing ME. Specific targeting of the EBV lytic phase protein BNLF2a to the transporter associated with antigen processing results in impairment of HLA class I-restricted antigen presentation. J Immunol. 2009;182(4):2313–24. https://doi.org/10.4049/jimmunol.0803218.
18. Lipińska AD, Koppers-Lalic D, Rychłowski M, Admiraal P, Rijsewijk FA, Bieńkowska-Szewczyk K, Wiertz EJ. Bovine herpesvirus 1 UL49.5 protein inhibits the transporter associated with antigen processing despite complex formation with glycoprotein M. J Virol. 2006;80(12):5822–32. https://doi.org/10.1128/jvi.02707-05.
19. Koppers-Lalic D, Verweij MC, Lipińska AD, Wang Y, Quinten E, Reits EA, Wiertz EJ. Varicellovirus UL 49.5 proteins differentially affect the function of the transporter associated with antigen processing, TAP. PLoS Pathog. 2008;4(5):e1000080. https://doi.org/10.1371/journal.ppat.1000080.
20. Ma N, Liu Z, Shi Z, Zhao XDM, Guo Z, Chen L. Bioinformatics Analysis of PRV proteins affecting TAP peptide transport. J Pathogen Biology. 2019;14(12):1386–93. https://doi.org/10.13350/j.cjpb.191205.
21. Liu H, Yang L. (2019). Functional analysis of prv-miR-LLT11a encoded by pseudorabies virus. 20(6), e68. https://doi.org/10.4142/jvs.2019.20.e68
22. Teisserenc H, Schmitt W, Blake N, Dunbar R, Gadola S, Gross WL, Cerundolo V. A case of primary immunodeficiency due to a defect of the major histocompatibility gene complex class I processing and presentation pathway. Immunol Lett. 1997;57(1–3):183–7. https://doi.org/10.1016/s0165-2478(97)00072-2.
23. Eggensperger S, ampé R. The transporter associated with antigen processing: a key player in adaptive immunity. Biol Chem. 2015;396(9–10):1059–72. https://doi.org/10.1515/hsz-2014-0320.
24. Halenius A, Hauka S, Dölken L, Stindt J, Reinhard H, Wiek C, Hengel H. Human cytomegalovirus disrupts the major histocompatibility complex class I peptide-loading complex and inhibits tapasin gene transcription. J Virol. 2011;85(7):3473–85. https://doi.org/10.1128/jvi.01923-10.

Publisher's note