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Antiserum to the recombinant truncated VP22 protein of herpes simplex virus type 1 that also recognizes full-length VP22

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Summary. – The herpes simplex virus type 1 (HSV-1) tegument protein VP22 encoded by the UL49 gene is essential for HSV-1 infection. However, its precise functions in the virus life cycle are unknown. A relatively important tool for disclosing these functions is an antiserum specifically detecting VP22 in the infected cell. To this end, a recombinant truncated VP22 protein consisting of C-terminal 45 aa fused to EYFP (enhanced yellow fluorescent protein) and His-tag was expressed in *Escherichia coli*, purified by the Ni^{2+}-NTA affinity chromatography, and used for the preparation of antiserum in rabbits. Western blot and immunofluorescence assay showed that this antiserum specifically detected purified truncated VP22 as well as full-length VP22 in the HSV-1 infected cells. These results indicate that the prepared antiserum could serve as a valuable tool for further studies of VP22 functions.

Keywords: herpes simplex virus type 1; truncated VP22; E. coli; recombinant protein; EYFP; antiserum

Introduction

HSV-1 infects mucocutaneous membranes and it is a relevant pathogen for the herpetic encephalitis, hepatitis, pneumonia, esophagitis, and keratitis. VP22 protein containing 301aa is the most abundant tegument protein of HSV-1 and conserved among the members of the subfamily *Alphaherpesvirinae* (Heine *et al*., 1974; Schlegel and Blaho, 2009). As an essential tegument protein. VP22 plays many important roles in both transiently transfected and HSV-1-infected cells, such as facilitating virion packaging, modulating transcription of the viral genes and enhancing the accumulation of viral proteins and mRNAs (Duffy *et al.*, 2009; Schlegel and Blaho, 2009; Yu *et al.*, 2010). Furthermore, VP22 has the capability of intercellular trafficking, binding to microfilaments, chromatin and nuclear membrane, reorganization of cytoskeleton, and nuclear and nucleolar localization (Harms *et al.*, 2000; Aints *et al.*, 2001; Li *et al.*, 2009). In addition, the interaction with HSV-1 proteins including VP16, gD and gE/gI and its association with host proteins such as template activating factor I and non-muscle myosin IIA also belong to the important characteristics of VP22 (Elliott *et al.*, 1995; Brignati *et al.*, 2003; van Leeuwen *et al.*, 2003; Chi *et al.*, 2005; O´Regan *et al.*, 2007a,b). However, the exact role of VP22 during HSV-1 infection remains unclear.

The aim of this study was a preparation of antiserum to the HSV-1 VP22 using a recombinant full-length VP22 tagged with His or both EYFP and His as an antigen. After our attempt to express this recombinant protein in *E. coli* failed, we succeeded with the expression of truncated recombinant protein consisting of C-terminal 45 aa. A rabbit antiserum to this protein recognized the truncated as well as full-length VP22.

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Abbreviations: EYFP = enhanced yellow fluorescent protein; FLAG = octapeptide DYKDDDDK; HA = hemagglutinin; HSV-1 = herpes simplex virus type 1; IF = immunofluorescence; IPTG = isopropyl-β-D-thiogalactoside; p.i. = post infection

Materials and Methods

Plasmid constructs. Primers for the construction of recombinant plasmids are listed in Table 1. Nucleotides encoding full length VP22, EYFP, or C-terminal 45 aa of VP22 were cloned into

Table 1. Primers for constructing of recombinant plasmids

Primer	Sequence (5′ to 3′)
HSV1-VP22-F	TGGAATTCATCTCTCGCCGCTC CGTG
HSV1-VP22-R-*Hin*d III	CGAAGCTTCTCGACGGCCGTCT GGGGC
HSV1-VP22- R-*Bam*H I	TTTGGATCCAACTACGCCGTCT GGGGCG
HSV1-VP22-C45-F	CGGAATTCATGGAGTTGGAATC CAGACG
YFP- F	CGGGATCCATGGTGAGCAAG GGCGAGGAG
YFP-R	CCGCTCGAGCTTGAAGCTCGTC CATGC

pET-28a (+) (Novagen), pEYFP-N1 (Clontech) or pCMV-N-HA, and pCMV-N-FLAG (Beyotime) to generate pET28a-VP22 and pET28a-EYFP, pVP22-EYFP and pC45-EYFP, or pCMV-HA-VP22 and pCMV-FLAG-VP22, respectively. Nucleotides encoding VP22-EYFP and C45-EYFP were subcloned into pET-28a (+) to yield pET28a-VP22-EYFP and pET28a-C45-EYFP, respectively.

Expression and purification of recombinant protein. *E. coli* were transformed with the recombinant plasmids (pET28a-VP22, pET28a-VP22-EYFP, pET28a-C45-EYFP, and pET28a-EYFP). The expression of recombinant proteins was analyzed by SDS-PAGE. The purification of recombinant protein was performed as described previously (Zhao *et al*., 2010).

Antiserum to the recombinant protein. The preparation of an antiserum was performed as described previously with the exception that the recombinant truncated VP22-EYFP-His protein was used in this study (Zhao *et al*., 2010).

Western blot analysis. Western blot analysis was performed as previously described with the exception that the antiserum against recombinant truncated VP22 was used in this assay (Zhao *et al*., 2010).

IF assay. IF assay was performed as previously described with the exception that the antiserum against recombinant truncated VP22 was used in this study (Pomeranz and Blaho, 1999).

Results and Discussion

Expression and purification of recombinant truncated VP22-EYFP-His protein

SDS-PAGE analysis showed that the full-length VP22 recombinant protein could not be expressed in the induced *E. coli* cells, although we optimized the expression conditions of both induction temperature and IPTG concentration (Fig. 1a). The use of EGFP (enhanced green fluorescent protein) or its color mutants as EYFP have been used as probes to the proteins expressed in live cells (Chalfie *et al*., 1994). Therefore, beside His-tag, the full-length VP22 was also tagged with EYFP-tag. Unexpectedly, there was no fluorescence observed, when VP22 was tagged with EYFP. SDS-PAGE analysis also indicated that the expression of full-length VP22-EYFP-His protein failed (Fig. 1b, c). However, the positive control EYFP-His protein was efficiently expressed (Fig. 1b). The failed expression of the full-length VP22 in *E. coli* could be explained as follows: there are two AGG rare codons in VP22, which may couse premature termination of the synthesized protein, create translation errors and/or reduce expression level; VP22 can not be efficiently folded in *E. coli*, since bacteria lack the ability to perform some posttranslational modifications; VP22 recombinant proteins could be toxic to the *E. coli*. These results could explain why so many polyclonal antibodies were produced against the truncated VP22 expressed in *E. coli* (Hafezi *et al*., 2005; Yu *et*

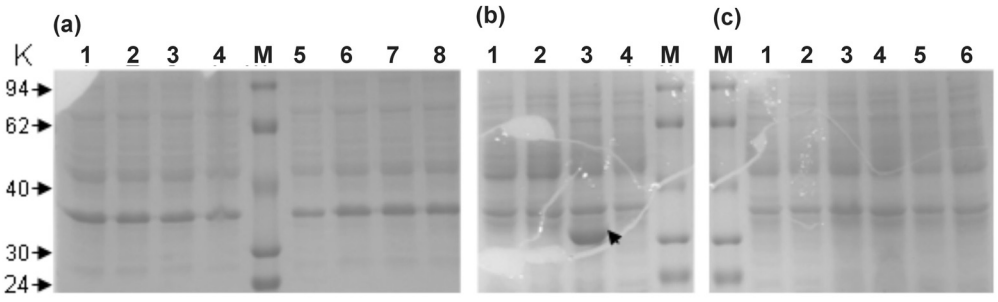

Fig. 1

SDS-PAGE analysis of full-length VP22 expression

(a) *E. coli* transformed with empty plasmid (negative control, lane 1) or plasmid encoding full-length VP22 protein and uninduced (lane 2) or induced with 1 mmol/l IPTG at 30°C (lane 3) or 37°C (lane 4) or 0, 0.5, 1.0 and 1.5 mmol/l IPTG at 37°C (lanes 5–8, respectively). (b) *E. coli* transformed with plasmid encoding full-length VP22-EYFP-His protein and induced (lane 1) or uninduced (lane 2) with IPTG, *E. coli* transformed with plasmid encoding EYFP-His and uninduced (lane 4) or induced with IPTG (positive control, lane 3); (c) *E. coli* transformed with plasmid encoding full-length VP22-EYFP-His protein and induced with 0, 0.5, 1.0, 1.5, 2.0, and 2.5 mmol/l IPTG at 37°C (lanes 1-6). Protein size marker (lanes M). Arrowhead indicates EYFP.

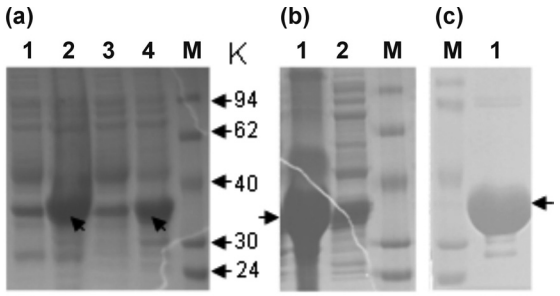

Fig. 2

Expression and purification of truncated VP22 analyzed by SDS-PAGE

(a) *E. coli* transformed with plasmid encoding truncated VP22-EYFP-His protein and grown at 30°C or 37°C and uninduced (lanes 1 and 3, respectively) or induced with 1 mmol/l IPTG (lanes 2 and 4, respectively). (b) Soluble (lane 1) and insoluble (lane 2) fractions from the sample shown on lane 4. (c) Purified truncated VP22 protein (lane 1). Protein size marker (lanes M). Arrowheads indicate truncated VP22.

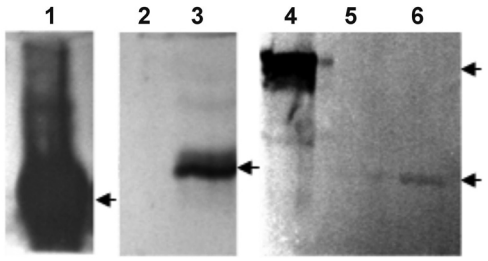

Fig. 3

Western blot analysis of truncated VP22 purification, HSV-1-infected cells, and VP22 recombinant protein-transfected cells

The antiserum against truncated VP22 protein was used. Purified truncated VP22-EYFP-His (lane 1), uninfected (lane 2), and infected (lane 3) Vero cells. HEK293T cells transfected with plasmid encoding full-length VP22-EYFP protein (lane 4), full-length VP22-FLAG (lane 5), and full-length VP22-HA protein (lane 6). Arrowheads indicate full-length VP22 protein.

al., 2010), although monoclonal antibody P43 against the full-length VP22 extracted from capsid/tegument structures was reported (Elliott and Meredith, 1992).

Due to the fact that the C-terminal region of VP22 is crucial for many functions of the VP22 (Elliott and O´Hare, 1997; Elliott *et al.*, 1999; Aints *et al.*, 2001; Martin *et al.*, 2002; Hafezi *et al.*, 2005; Schlegel and Blaho, 2009; Yu *et al.*, 2010), the truncated VP22 region (257–301aa) fused to EYFP and His-tag was expressed. SDS-PAGE analysis showed that the truncated VP22-EYFP-His recombinant

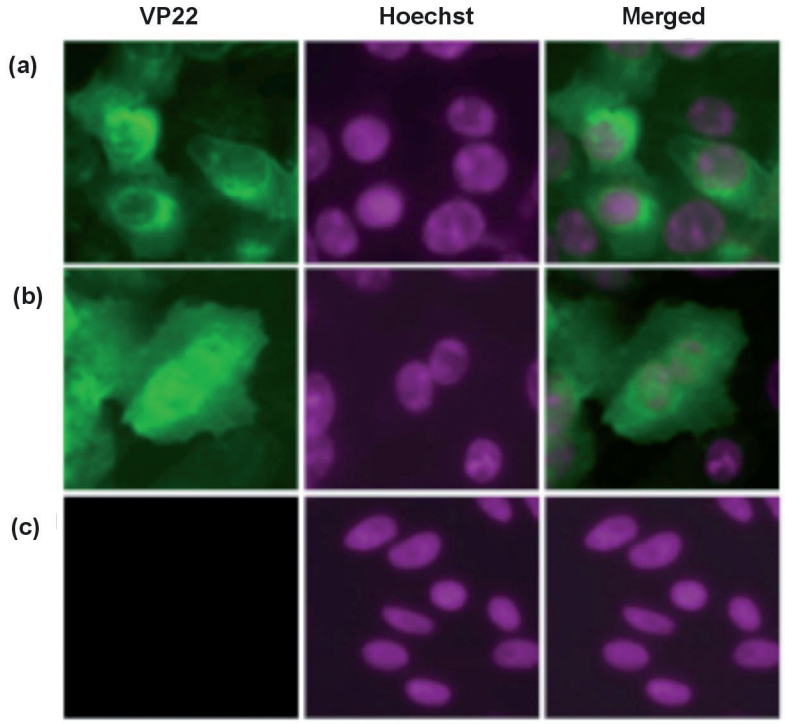

Fig. 4

IF assay of the native VP22 protein in HSV-1-infected Vero cells

Uninfected (c) and infected cells at 7 (a) and 9 (b) hrs p.i. were probed with the antiserum against truncated VP22. Cells were labeled with FITC-conjugated goat anti-rabbit immunoglobulin G and counterstained with Hoechst stain to visualize the nuclei.

protein exhibited a very high level of expression in *E. coli* (Fig. 2a). Furthermore, SDS-PAGE analysis of the soluble fraction and cell debris showed that the majority of the induced protein was found in the soluble fraction of cells (Fig. 2b). The analysis also showed adequate purity of the expressed protein (Fig. 2c).

Antiserum against recombinant truncated VP22-EYFP-His protein

The rabbit antiserum against the truncated VP22-EYFP-His protein was collected after 3 injections of the antigen. Western blot analysis demonstrated that the prepared antiserum specifically recognized the purified truncated protein (Fig. 3). In addition, the antiserum detected also native VP22 present in the HSV-1-infected Vero cells (Fig.3). However, no protein was detected in mock-infected Vero cells (Fig. 3). The antiserum could also be used for the detection of differently tagged full-length VP22 recombinant proteins, such as the full-length VP22-EYFP, full-length VP22-FLAG, and full-length VP22-HA protein (Fig.3). The subcellular localization of native VP22 during HSV-1 infection was further examined by IF assay using the prepared antiserum. Obtained results showed that the VP22 protein exhibited a cytoplasmic localization in the infected cells at 7 hrs p.i. (Fig. 4a). Later in the infection cycle at 9 hrs p.i., the protein was detected in the cytoplasm as well as in the nucleus of HSV-1-infected cells (Fig. 4b), which resembled the localization pattern reported in previous work (Pomeranz and Blaho, 1999). However, there was no staining observed in the mock-infected cells (Fig. 4c).

Taken together, the antiserum against truncated VP22 could recognize the truncated as well as full-length VP22 protein. Therefore, this antiserum may serve as a useful tool for further studies of VP22 biological functions in the course of HSV-1 infection.

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