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Short communication

Equine herpesvirus type 1 affects mitochondrial network morphology and reactive oxygen species generation in equine dermal cell line

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Abstract

In the present study, the influence of the infection with equine herpesvirus type 1 (non-neuropathogenic and neuropathogenic strains of EHV-1) on the morphology and distribution of mitochondrial network in equine dermal cell line was investigated. Our results indicate that EHV-1-infection caused changes in the mitochondrial morphology manifested mostly by fission and reactive oxygen species generation.

Key words: equine herpesvirus type 1, equine dermal cell line, mitochondria, reactive oxygen species

Introduction

Equine herpesvirus type 1 (EHV-1) is one of the most dangerous pathogens of horses causing respiratory diseases, abortion and neurological disorders. In previous studies, we have demonstrated that EHV-1 affects structures of the cytoskeleton in equine dermal cells (ED) (Turowska et al. 2010), however no information concerning the effects of EHV-1 on the mitochondrial network in these cells is available. It is worth noting, that any disturbances in the dynamics of the mitochondrial network and its function suggest, that they can represent one of the viral strategies aimed at disrupting cellular signalling pathways, that induce an immune response (Seth et al. 2005, Khan et al. 2015). So far little is known about strategies used by viruses to disrupt the function of mitochondria during infection. Therefore, in the present study we investigated the effect of EHV-1 infection on the organisation of the mitochondrial network and its function in equine dermal cells.

Materials and Methods

Cell culture and virus strains

ED cell line (ATCC CCL57) was grown in Eagle's minimum essential medium (MEM) with Earle's salts, 5% of inactivated foetal bovine serum and 40 mg/ml of gentamicin (Gibco Life Technologies) at 37°C with 5% CO_2. Three different EHV-1 stains were used:

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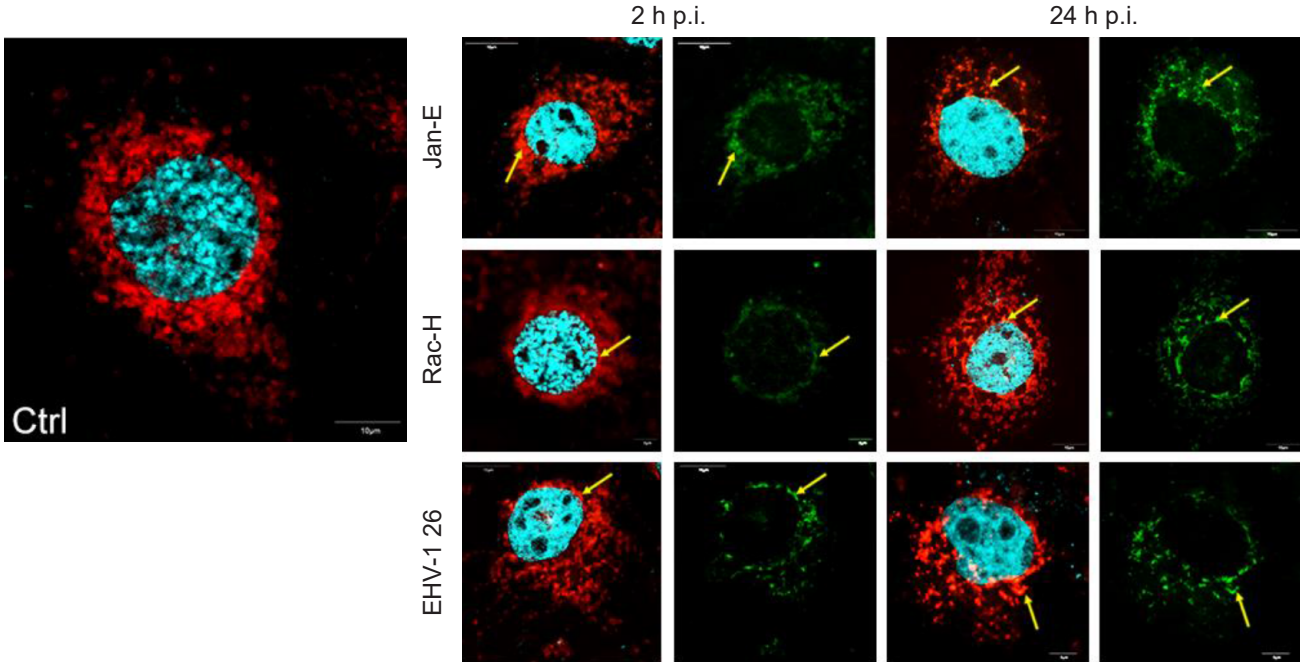

Fig. 1. Mitochondrial network morphology in equine dermal cells during EHV-1 infection. Representative confocal images of ED cells obtained at 2 and 24 h p.i. with all strains of EHV-1. Arrows indicate colocalization of viral antigen and mitochondria. Mitochondria – red fluorescence, viral antigens – green fluorescence, nuclei – blue fluorescence. Objective magnification x 60.

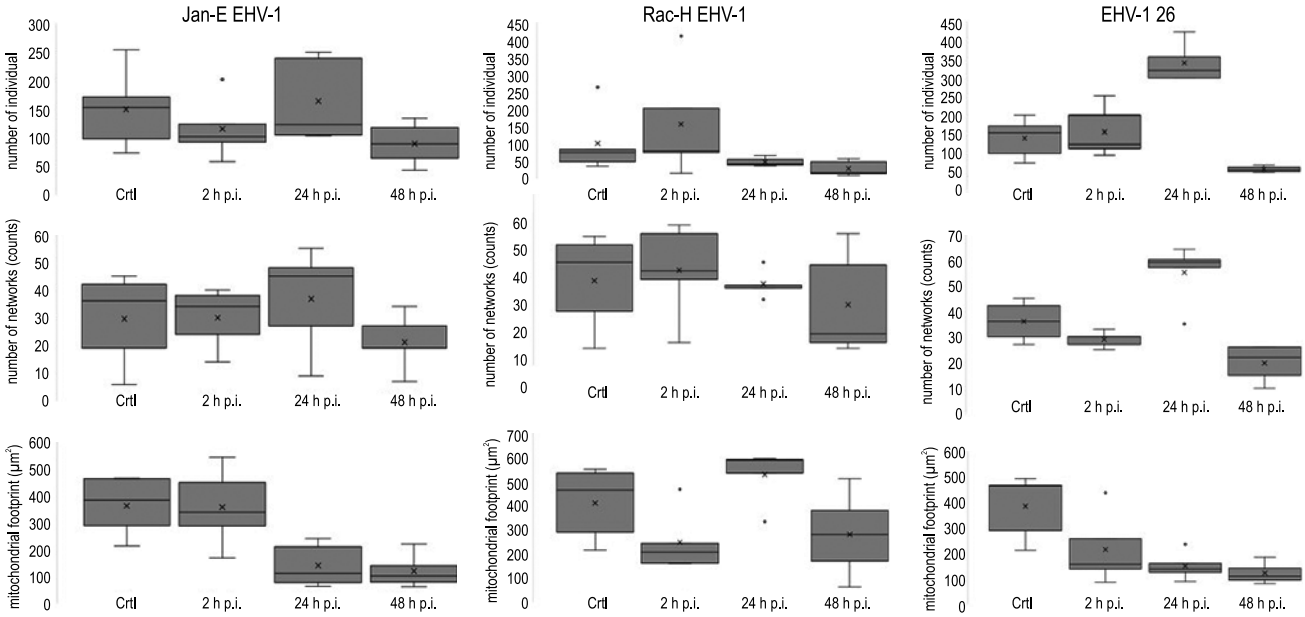

Fig. 2. Characterization of mitochondrial morphology of ED cells during EHV-1 infection with MiNa Single Image Macro Tools. The number of individuals, number of networks and mitochondrial footprint were examined. The graph shows a summary statistic of infected ED cells and control cells (each analysis was performed on 10 cells). Box plot show median (horizontal lines), first to third quartile (box), and the most extreme values (%). Statistical differences were interpreted as significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

(i): non-neuropathogenic, reference strain Rac-H; (ii) non-neuropathogenic Jan-E strain isolated from aborted fetus (neuropathogenicity confirmed by PCR-RFLP neuropathogenic/non-neuropathogenic discrimination test) (Cymerys et al. 2016); (iii) neuropathogenic EHV-1 strain (EHV-1 26) isolated from aborted fetus in Hungary in 2004 (neuropathogenicity confirmed by PriProET technique) (Malik et al. 2010). ED cells were infected with Rac-H, Jan-E or EHV-1 26 strains (MOI = 1.0) for 60 min at 37°C. After incubation, the inoculum was removed and fresh culture medium was added. Subsequently, infected cells were incubated for 2, 24 and 48h at 37°C with 5% CO_2.

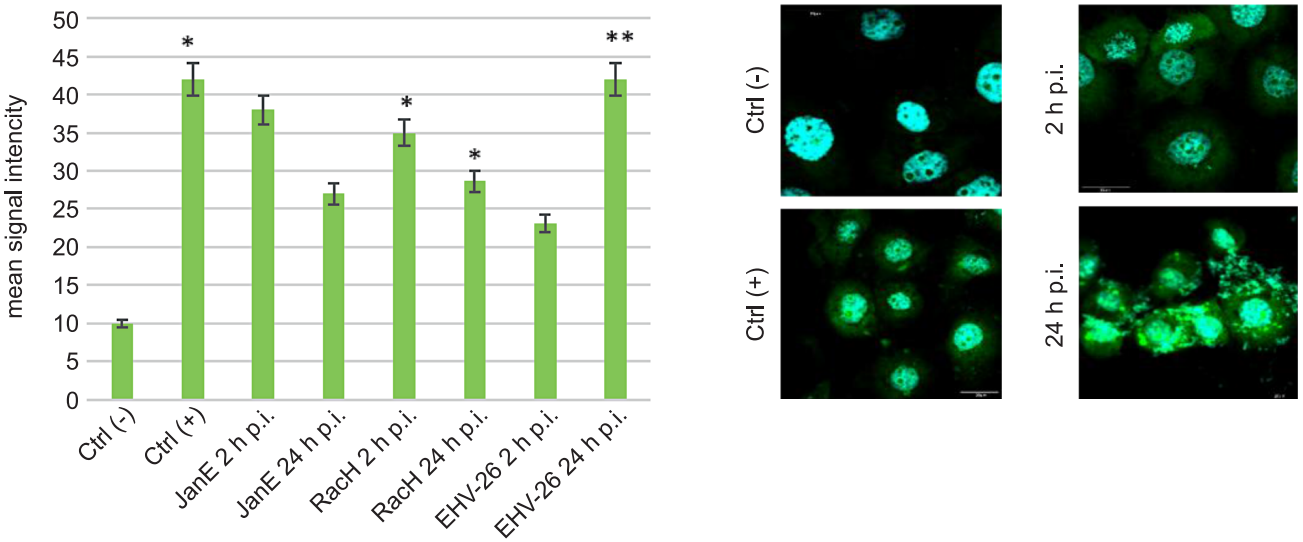

Fig. 3. The level of reactive oxygen species (ROS) in ED cells during EHV-1 infection (2 and 24 h p.i.), measured with the CellROX Green Reagent. Uninfected cells treated with 1 mMH_2O_2 were used as a positive control. (%). Statistical differences were interpreted as significant at p≤0.05 (*) and p≤0.01 (**). Representative images of confocal analysis showed generation of ROS in ED cells during Jan-E EHV-1 infection. ROS – green fluorescence, nuclei – blue fluorescence. Objective magnification x 60.

Immunofluorescent staining procedure

To visualize mitochondria, ED cells seeded on glass coverslips in a 6-well plate were incubated with 100 nM MitoRed (Sigma-Aldrich) for 30 min at 37 °C. Afterwards, the cells were fixed in 3.7% paraformaldehyde/ /PBS (Sigma-Aldrich) and the presence of viral antigen was detected by direct immunofluorescence, using polyclonal antiserum EHV-1/ERV conjugated to FITC (VMRD, Inc.). Cell nuclei were stained with Bisbenzimidine/Hoechst 33258 (Hoechst AG) according to the manufacturer's recommendations. Reactive oxygen species (ROS) levels were measured with the CellROX Green Reagent (Thermo Fisher Scientific), a fluorogenic probe for measuring oxidative stress in live cells (Ex/Em~485/520 nm). Control and EHV-1-infected cells were stained with 5 µM CellROX Green Reagent and Hoechst 33342 by adding the probe to the complete media and incubating at 37 °C for 30 min. The results were evaluated using a confocal microscope (Fluoview FV10i, Olympus) with a 60x water-immersion lens. For analysis of the mitochondrial morphology, MiNa Single Image Macro Tools (Fiji, version: 2019) was used according to the protocol of Valente et al. (2017).

Results and Discussion

Immunofluorescent labelling and confocal image analyses revealed the presence of mitochondria in the perinuclear area of the EHV-1-infected cells and its colocalization with viral antigen (Fig. 1, arrows). EHV-1 infection led to the fission of the mitochondrial network, which was manifested by the increase in the number of punctate mitochondria. These results were confirmed by MiNa Macro Tools analysis (Fig. 2). As a consequence of the infection, a decrease in the total mitochondrial area and number of branched mitochondria in ED cells was observed. Moreover, EHV-1 infection induced ROS generation in ED cells, with the highest increase detected for the neuropathogenic strain (EHV-1 26) at 24 h p.i. (hours post infection) (Fig 3). These results indicated that increased production of ROS may be an additional factor contributing to the destruction of the mitochondrial network. Similar results were obtained during the analysis of the mitochondrial network distribution in cultured human keratinocytes infected with the HHV-1 and HHV-2 virus examined by Chodkowski et al. (2018). Total defragmentation of the mitochondrial network occurred after 48 hours post infection, at the time of intense viral replication. In addition, colocalization of the viruses with the mitochondrial network was determined (Chodkowski et al. 2018).

In conclusion, our results suggest that mitochondria play the crucial role during EHV-1 infection. The changes observed in the organization of the mitochondrial network and physiology of infected ED cells provide appropriate conditions for EHV-1 replication and are required for effective viral spread.

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