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Letter

Migrasomes released by HSV-2-infected cells serve as a conveyance for virus spread

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Dear Editor,

Migrasomes are newly discovered cellular organelles with diameters of 0.5–3 μm which have been found to be produced by normal and cancer cells, and distributed in various organs of animals (Ma et al., 2015) and in human sera (Zhao et al., 2019). Migrasomes are present inside the cavities of pulmonary alveoli, blood vessels and lymph capillaries (Zhang et al., 2020), and can be captured by surrounding cells with their cargoes internalized. The biological roles of migrasomes have been reported in cell-to-cell communication (Zhu et al., 2021), organ morphogenesis during zebrafish embryonic development (Jiang et al., 2019), embryonic angiogenesis (Zhang et al., 2022a) and maintenance of cellular homeostasis (Jiao et al., 2021). Furthermore, migrasomes have been used as a diagnostic marker of early renal system injury in patients with diabetic-nephropathy (Liu et al., 2020). Recently, Zhang et al. reported that Chikungunya virus nsP1 can induce the formation of migrasomes (Zhang et al., 2022b); however, whether migrasomes play a role in virus infections remains unknown. It is unclear whether cells infected with viruses can release migrasomes containing virions. If so, it is unknown whether such migrasomes act as intermediate carriers for virus transmission. Herpes simplex virus type 2 (HSV-2), a typical member of the *α-herpesvirus* subfamily, can infect genital epithelium to cause herpes (Kimberlin and Whitley, 2007). HSV-2 infection also increases the risk of HIV-1 acquisition and transmission (Baeten et al., 2004; Freeman et al., 2006). Currently known modes of HSV-2 transmission include virus-cell and cell-to-cell spread mediated by the virus and virus-infected cells, respectively (Sattentau, 2008). Whether HSV-2 uses other mechanisms to spread remains elusive. Considering the characteristics of migrasomes and the fact that HSV-2 spread is predominantly dependent on cell-to-cell contact, we speculated that HSV-2 may take advantage of migrasome as a "Trojan horse" to be transmitted from cell to cell as one of the spread routes (Schematic diagram in Fig. 1A).

To test this hypothesis, HSV-2 was used as a model to infect HaCaT cells in the culture dishes pretreated with fibronectin (FN). Migrasomes were collected at 20 h post infection (hpi) and purified by ultracentrifugation (Supplementary Fig. S1). The contents of the isolated migrasomes were observed under electron microscopy. As shown in Fig. 1B, HSV-2 virions were found in the migrasomes. Considering the conventional use of tetraspanin-4 (TSPAN4), carboxypeptidase Q (CPQ) and bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1 (NDST1) for migrasome detection (Bassani and Cingolani, 2012; Huang et al., 2019), HaCaT cells infected with HSV-2 were fixed with polyformaldehyde at 20 hpi, followed by immunofluorescence assay. Colocalization of the migrasome markers TSPAN4, CPQ and NDST1 with the major capsid protein ICP5 of HSV-2 in the extracellular vesicle was detected using immunofluorescence (indicated by the red arrows in Fig. 1C). In addition, ultrathin sections of HSV-2 infected mouse tissues were observed under transmission electron microscope (TEM) and fluorescence microscope. Six to eight-week-old female C57BL/6J mice were challenged intravaginally (i.vag.) with HSV-2 (G strain). The mice were sacrificed when genital ulceration and severe inflammation were observed at 7 dpi. The intestinal and cervical tissues were collected for TEM analysis and immunofluorescence staining. TEM images showed that HSV-2 virions exist in the migrasome-like structure of mouse intestinal (Fig. 1E) and cervical (Fig. 1G) tissues. The colocalization of TSPAN4 and ICP5 in the vesicle was detected in the mouse intestinal (indicated by white arrows in Fig. 1D) and cervical tissues (indicated by white arrows in Fig. 1F). These findings support our hypothesis that HSV-2 virions can be encapsulated in migrasomes.

We then assessed whether HSV-2 in the isolated migrasomes could be transmitted to uninfected cells and cause productive infection. HSV-2-GFP was used to infect HaCaT cells in the culture dish pretreated with fibronectin (FN). Migrasomes were subsequently isolated at 20 hpi and purified by ultracentrifugation. Cell-free HSV-2-GFP supernatant was filtered with 0.45 μm filter and the purified migrasomes were added into culture dishes coated with HSV-2 permissive cells (HaCaT) or non-permissive cells (CHO). As shown in Fig. 1H, both cell-free HSV-2-GFP

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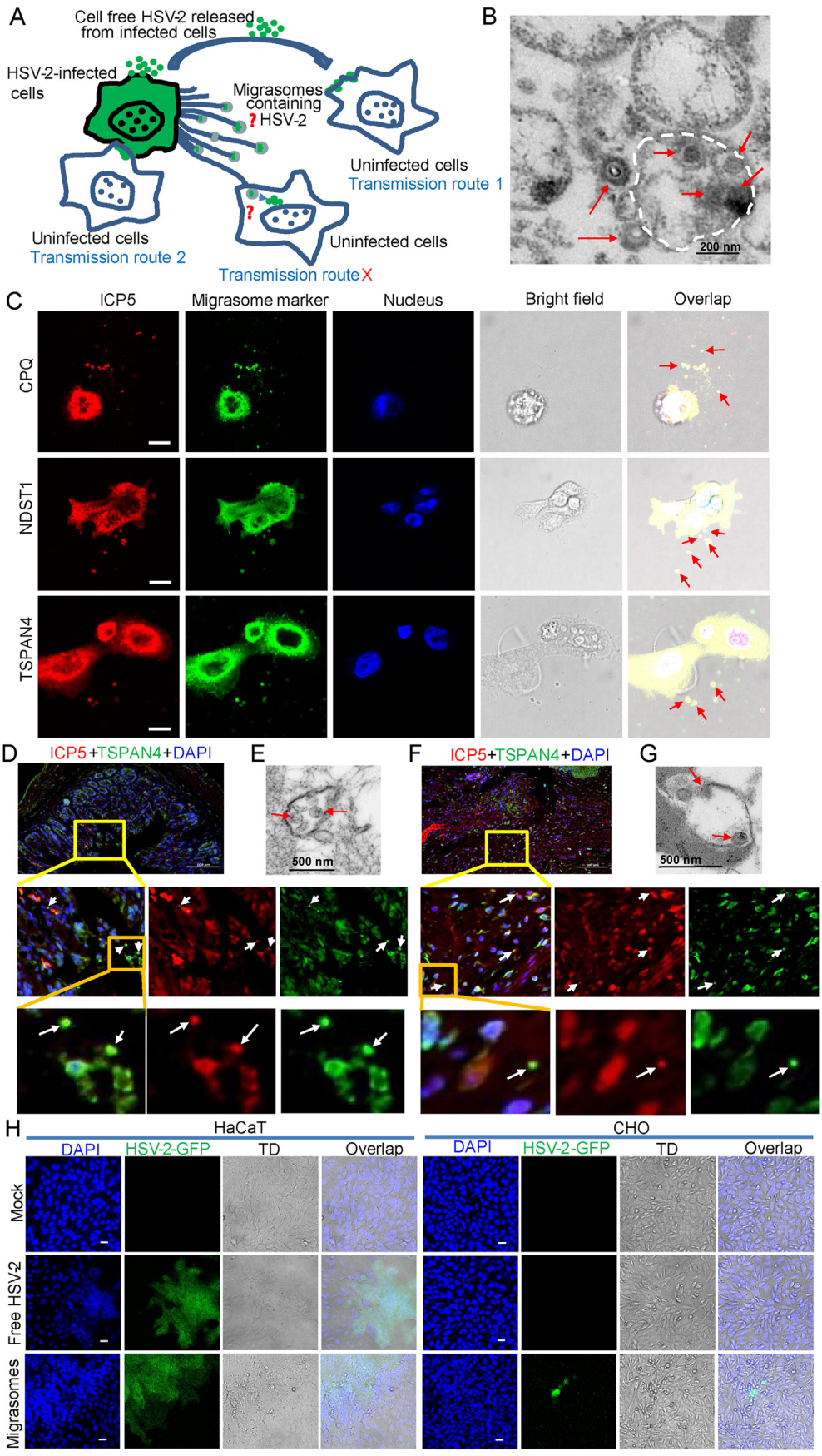

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Fig. 1. Migrasomes facilitate HSV-2 cell-to-cell spread. **A** Schematic diagram of HSV-2 transmission routes. **B** TEM image of HSV-2 virions in an isolated migrasome fraction. Scale bar, 200 nm. **C** Colocalization of migrasome markers CPQ, NDST1 and TSPAN4 with HSV-2 ICP5 in HSV-2-infected HaCaT cells was detected under fluorescence microscope. HaCaT cells infected with HSV-2 were fixed with polyformaldehyde at 20 hpi, followed by immunofluorescence assay. Scale bar, 10 μm. **D** TSPAN4 and HSV-2 ICP5 in ultra-thin sections of mouse intestinal tissues were assessed by immunofluorescence. Scale bar, 100 μm. **E** Migrasomes containing HSV-2 in ultra-thin sections of mouse intestinal tissues were observed under TEM. Scale bar, 500 nm. **F** TSPAN4 and HSV-2 ICP5 in ultra-thin sections of mouse cervical tissues were assessed by immunofluorescence. Scale bar, 100 μm. **G** Migrasomes containing HSV-2 in ultra-thin sections of mouse cervical tissues were observed under TEM. Scale bar, 500 nm. **H** Fluorescence images of HSV-2 infected HaCaT or CHO cells. Scale bar, 50 μm. One representative experiment out of three is shown. All experimental details were provided in Supplementary Materials.

and purified migrasomes resulted in the infection of HaCaT cells with green plaques and syncytia being observed. No green fluorescence was observed in HSV-2 non-permissive CHO cells following the infection of cell-free HSV-2-GFP. In contrast, although no plaque or syncytium formation was detected, green fluorescence was seen in individual CHO cells co-cultured with purified migrasomes, suggesting that HSV-2-GFP spread to CHO cells likely via migrasomes, which could lead to productive infection.

In conclusion, the results of this study suggest a novel mechanism of HSV-2 cell-to-cell spread mediated by migrasomes, providing a potential intervention target against HSV-2 transmission. To our knowledge, this is the first time that migrasomes have been found to play a role in virus spread.

Footnotes

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