Live-Cell Real-Time Imaging of Mitochondria in HIV-1-Infected Cells

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FIG. 1. Live-cell real time fluorescent image in DAP-RFP-GFP overlay of a coculture of HIV-1-infected H9 cells and uninfected target cells. The HIV-infected H9 cells are fluorescently labeled with MitoTracker Red CMXRos to stain mitochondria *red* and Hoechst 33342 to stain nuclear DNA *blue*. The uninfected MT2 target cells express GFP (*green*). Polarization of mitochondrial location (*red*) is seen, as mitochondria accumulate at the junction between the H9 cell and the MT2 cell. Mitochondria from H9 cells are seen in the MT2 cells as *yellow* staining (*red on green*). The *insert* in the *top right corner* represents a typical field of the coculture. Cell-to-cell viral transmission and target cell infection bring about characteristic HIV-1 cytopathological effects, including formation of virological synapses and syncytia. The figure shows the area in the field indicated by the *white arrow*.

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HIV-1 IS CAPABLE OF INFECTING target cells by both cell-free viral infection and cell-to-cell transmission.¹ In cell-free viral infection, free virus is exposed to cellular, immunological, and drug barriers, so infection efficiency is low and drug sensitivity is high. In cell-to-cell transmission, virus is transmitted directly from cell to cell, shielded from most of the barriers. Thus, the infection efficiency is much higher, and drug sensitivity is lower. Importantly, HIV spreads *in vivo* mainly by cell-to-cell transmission. In contrast to cell-free viral infection, the mechanism of cell-to-cell HIV transmission is much less understood. At present, there is no drug to cure AIDS and no vaccine to prevent HIV infection. Viral mutation, viral latency, epitope specificity/accessibility, and mechanism of viral transmission all contribute to difficulties in prevention and cure.²

Mitochondria have long been known to be essential organelles. In addition to providing metabolic energy, they are involved in many cellular signaling pathways, including host innate immunity, apoptosis, autophagy, and aging.³ We used live-cell, real-time fluorescence imaging of cocultures of HIV-1-infected T cells and uninfected target cells to investigate the role of mitochondria in cell-to-cell HIV transmission.⁴ We reported that mitochondria of HIV-infected cells serve as viral reservoirs and carriers, carrying virus into target cells, facilitating viral transmission, and advancing cell-to-cell viral spread. Furthermore, we reported that cell-free mitochondria purified from HIV-1-infected cells are infectious.⁴

Figure 1 represents a live-cell real-time fluorescent image in *blue-red-green* (DAP-RFP-GFP) overlay of a coculture of HIV-infected H9 T cells and uninfected GFP-MT2 target cells. The HIV-infected H9 cells are fluorescently labeled with MitoTracker Red CMXRos to stain mitochondria *red* and Hoechst 33342 to stain nuclear DNA *blue*. The uninfected MT2 target cells express GFP (*green*). Polarization of

mitochondrial location (*red*) is seen, as mitochondria accumulate at the junction between the H9 cell and the MT2 cell. Mitochondria from H9 cells are seen in the MT2 cells as *yellow* staining (*red on green*). These results provide further insight into intercellular interactions previously reported.⁴

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Author Disclosure Statement

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