ROLE OF TETRASPANINS IN HTLV-1 BIOFILM FORMATION

Arone C[1], Dutartre H[2], Muriaux D[1]*
[1] Institut de Recherche en Infectiologie de Montpellier (IRIM – Montpellier, France), [2] Centre International de Recherche en Infectiologie (CIRI – Lyon, France), *: corresponding author

Background
With 5 to 10 million people infected worldwide, the Human T-Lymphotropic Virus type-I (HTLV-1), mainly detected in CD4+T-lymphocytes, represents a global health issue as no therapeutic approach exists to protect exposed individuals. HTLV-1 is almost exclusively transmitted during inter-cellular contacts through two predominant routes: virological synapses and viral biofilms. The latter are adhesive structures polarized on the cell surface that confine virions in a protective environment and allow their simultaneous delivery during infection. Our work aims to pursue the molecular characterization of these viral biofilms and identify the mechanisms ruling their formation.

Methods
Our approach is based on a large-scale identification of proteins contained in HTLV-1 biofilms isolated from chronically infected T-lymphocytes. The expression pattern of interesting protein candidates and their presence in HTLV-1 biofilms were analyzed by immunofluorescence coupled with confocal or stimulated-emission-depletion (STED) microscopy. shRNA silencing was performed to conclude on candidate molecules' function. ImageJ/Ilastik software were used for image analysis.

Results
Mass spectrometry analysis revealed enrichment of several tetraspanins, transmembrane proteins that organize microdomains on cell membranes, in released HTLV-1 biofilms. Interestingly, CD9 and CD82, known to cluster into HTLV-1 biofilms, but also CD81 were detected. Accordingly, we obtained a strong polarization of these tetraspanins in cell-attached biofilms by immunofluorescence, especially CD81 for which 40% of its total signal overlapped with fluorescent biofilms. Using STED microscopy, we also noted a 3-fold increase in CD81 platforms area at biofilms positions. Finally, CD81 shRNA silencing induced a 2-fold increase of cell-associated HTLV-1 Gag and a decrease in viral release, suggesting potential roles for CD81 in HTLV-1 biofilms formation, viral release and/or biofilms detachment.

Conclusion
Altogether, these results suggest an enrichment of several tetraspanins including CD81 into biofilms and a potential role of these molecules in viral clustering, biofilm adhesion, and biofilm polarization, which are essential for HTLV-1 transmission.

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