HIV-1 concentrates and shelters cell-associated infectivity a “viral biofilm”

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understand the mechanisms through which incentives may increase and sustain retention in HIV services.

TUAD0205

Using conjoint analysis to model hospital directors' decision-making in adoption of an evidence-based stigma-reduction intervention

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Background: Behavioural interventions that have demonstrated efficacy in randomized trial conditions have been underutilized in healthcare delivery. This study used conjoint analysis, a marketing research technique, to quantify the impact of different aspects of intervention in hospital stakeholders' decision-making in adoption of evidence-based interventions (EBI).
Methods: The authors used a real-life intervention with efficacious outcome to reduce HIV-related stigma in healthcare settings as a "product" to study adoption of EBI. Conjoint analysis was conducted among 60 hospital directors recruited from 30 hospitals of different levels and types in Fujian Province, China. The directors evaluated their willingness to adopt the evidence-based stigma reduction intervention in their hospitals by rating across eight hypothetical scenarios with preferred and non-preferred levels of seven attributes, including (1) administrative support, (2) cost, (3) personnel involvement, (4) format, (5) duration, (6) technical support and (7) priority alignment with the hospital. A mixed-effect model was fit to the likelihood of intervention adoption for the eight scenarios, and the seven attributes (categorized as preferred = 1 or not preferred = 0) served as independent variables in the model.
Results: Monetary cost of intervention implementation (impact score = 24.8) had the greatest impact on the directors' willingness to adopt a certain EBI, followed by duration of the intervention (impact score = 10.0), availability of technical support (impact score = 7.5) and flexibility of format (impact score = 4.6). The majority (88.3%) of the hospital directors perceived the conjoint administration process as clear and easy to understand. The data collection time was relatively short, which was approximately 30 minutes.
Conclusions: Conjoint analysis was proven to be feasible in modelling hospital directors' decision-making in adoption of EBI. There were several issues that one should consider when operationalizing conjoint analysis in dissemination and implementation research, including selection of EBI example, assigning the component level of the attributes, generating scenarios and interviewer training. The findings have implications for design and dissemination of existing EBI in healthcare settings to optimize the public health impact.

WEAA0101

Impaired Nef's ability to counteract SERINC5 is associated with decreased plasma viraemia

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Background: It is recently revealed that an HIV-1 accessory protein Nef plays an essential role in virion infectivity by antagonizing a host restriction molecule SERINC5. However, it remains elusive whether Nef's ability to counteract SERINC5 influences viral fitness *in vivo*. Because Nef is a highly polymorphic protein due to the selective forces by host cellular immunity, we hypothesized that certain immune-escape polymorphisms might affect the Nef function and thereby plasma viraemia.
Methods: We collected plasma viral RNA from HLA-typed, treatment-naive, chronically HIV-1-infected subjects (n = 375) and analysed DNA sequences of Nef-encoding region. Immune-associated Nef polymorphisms were analysed by a phylogenetic network model. We also introduced several mutations to a control strain and patient-derived Nef clones and tested various Nef functions *in vitro*, including downregulation of CD4 and HLA class I as well as enhancement of virion infectivity and counteraction of SERINC5.
Results: We identified 112 Nef polymorphisms that were overrepresented within patients sharing the same HLA genotypes. Specifically, two mutations, Tyr-120 to Phe and Gln-125 to His, were overrepresented in patients carrying HLA-B*51:01 and HLA-C*14:03, and the number of the two mutations correlated inversely with plasma viral load (p = 0.004). Nef functional assays demonstrated that the double-mutant Nef impaired in SERINC5 counteraction and enhancement virion infectivity whereas other Nef functions such as CD4 and HLA class I downregulation remained unchanged. Jurkat cells lacking SERINC5 expression lost such functional difference between the parental and mutant Nef clones.
Conclusions: Taken together, these results suggest that naturally occurring immune-associated mutations impair Nef's ability to counteract SERINC5 and enhance virion infectivity, associating with reduced plasma viral load *in vivo*.

WEAA0102

HIV-1 concentrates and shelters cell-associated infectivity a "viral biofilm"

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Background: Highly active antiretroviral therapy (HAART) does not allow the complete clearance of the virus since it does not target viral reservoirs nor efficiently block HIV-1 cell-to-cell transmission *in vivo*. HIV-1 cell-to-cell spread is thousands-fold more efficient than cell-free infection; yet, how virions are transferred *via* cell contacts remains unknown.
Methods: Using a panel of cutting-edge imaging techniques (Cryo-TEM, CL/SEM, CL/FIB and super-resolution imaging) to functional assays, we investigated and characterized viral structures involved in HIV-1 cell-associated infectivity. We analysed a range of infected T-cell cultures (chronically infected T-cell lines, primary CD4+ T cells infected *in vitro* with virus primary isolates and CD4+ T lymphocytes from untreated HIV-1-infected patients with a detectable viraemia).
Results: We show here that HIV-1 cell-associated infectivity mostly resides at the surface of CD4+ T lymphocytes in a viral biofilm, formed by viral particles aggregated within a scaffold of

extracellular matrix (ECM) components. Our set of data demonstrates that (i) biofilm-associated viral particles are more efficient in establishing infection than free viral particles and (ii) they confer HIV with important properties characterizing cell-to-cell spread. This includes the decreased sensitivity to HAART and to the broad neutralizing antibody 3BNC117. HIV-1 regulates biofilm matrix composition that controls both viral particles organization at the cell surface and the resulting cell-associated infectivity. The organized clustering of viral particles along an ECM framework locally concentrates virions, favours their collective transfer to target cells and limits their exposure to nAbs. Finally, CD4+ T cells from HIV-1-infected patients produce and transmit viral biofilms, supporting that they may also be involved *in vivo*.

Conclusions: This study thus unveils HIV biofilm as a new highly infectious extracellular entity that concentrates, stores, disseminates and shelters HIV-1 infectivity. This may have implications for HIV-1 spread and persistence in host, including for maintaining HIV-1 sanctuaries in treated patients. Our results unveil a new role for the ECM in clustering and protecting HIV-1 at the plasma membrane and in their collective transfer through virological synapses. Targeting biofilm ECM components could represent a promising approach to favour HIV-1 clearance or to potentiate the effect of available anti-viral therapies.

WEAA0103

Coordinated mTOR-mediated rewiring of nucleotide anabolism regulates HIV-1 infection of CD4 T lymphocytes

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Background: HIV-1 replication is restricted in resting CD4 T lymphocytes. Stimulation of these cells via either the T-cell receptor (TCR) or gamma-cytokine receptors up-regulates HIV provirus formation at levels of reverse transcription (RT) and nuclear import. However, the enhancements of both RT and nuclear import are not fully explained by activation-induced changes in known restriction factors. Here, we study a "master regulator" activated by both TCR and gamma-cytokine signalling: the mechanistic target of rapamycin (mTOR) kinase.

Methods: Resting CD4 T cells purified from blood donor PBMCs using immunomagnetic cell separation were stimulated with anti-CD3/anti-CD28 beads, PHA/IL2 or IL7/15. Inhibitors were used before activation to study the role of mTOR. qPCR quantified HIV-1 RT products and 2-long terminal repeat (2-LTR) circles. Flow cytometry after infection with a single-round HIV-1-GFP reporter virus monitored productive infection. Quantitation of dNTPs used ultra-sensitive LC-MS/MS detection. Flow cytometry and immunoblotting assessed effects of treatments on mTOR activity.

Results: mTOR activity induced by engagement of either T cell or gamma-cytokine receptors coordinates expression of transporters for glucose (*GLUT1*), glutamine (*ASCT2*) and transferrin (*CD71*), as well as rate-limiting enzymes for pyrimidine (*CAD*), purine (*IMPDH2*) and deoxyribonucleotide (dNTP) synthesis (*RRM1*). mTOR has been previously reported to govern the expression of nutrient transporters and pyrimidine biosynthetic genes, but this is the first demonstration of this global mTOR-dependent programme in activated CD4 T lymphocytes. Pharmacological ablation of mTOR activity suppressed dNTP pool expansion after activation. Multiple chemically distinct catalytic inhibitors of mTOR were found to reduce HIV-1 RT products after TCR stimulation. Moreover, both TCR and gamma-cytokine-activation induced mTOR inhibitor-sensitive accumulation of 2-LTR circular forms of HIV-1 DNA, indicating that mTOR activity also regulates active, energy (GTP/ATP)-dependent HIV-1 nuclear import.

Conclusions: CD4 T lymphocyte activation-induced mTOR "metabolic reprogramming" drives increased susceptibility to HIV-1 by expanding key nucleotide substrate and energy pools necessary for both reverse transcription and nuclear import. This adds mechanistic understanding, confirms earlier reports that catalytic inhibitors of mTOR hold promise for improving HIV-1 chemotherapy and prevention and suggests continued investigation of mTOR's role in establishment as well as reactivation of HIV-1 infection.

WEAA0104

Membrane-associated RING-CH (MARCH) 1 and 2 are other members of MARCH proteins that inhibit HIV-1 infection

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Background: Membrane-associated RING-CH 8 (MARCH8), which is one of the 11 members of MARCH family, downregulates several host membrane proteins (MHC-II, CD86, transferrin receptor, etc.). We have recently reported that this protein also targets HIV-1 envelope glycoproteins and acts as an antiviral factor (*Nat. Med*. 21:1502–1507, 2015). It remains unclear whether other family members might have similar antiviral functions to those seen in MARCH8. Here, we show that MARCH1 and MARCH2 are such MARCH family members that reduce virion incorporation of envelope glycoproteins.

Methods: Plasmids expressing family members of MARCH (MARCH1, MARCH2, MARCH3, MARCH5, MARCH6 and MARCH7) were created by RT-PCR-amplifying their mRNAs. The HIV-1 proviral luciferase indicator plasmid was cotransfected with plasmids expressing either HIV-1 Env or VSV-G, and those expressing MARCH family members including MARCH8, into 293T cells. Supernatants were subjected to assays for infectivity, viral entry or virion incorporation, and producer cells were used for flow cytometry. Real-time RT-PCR was performed to determine endogenous levels of MARCH1 and MARCH2 expression.

Results: Infectivity assays showed that, two other members of MARCH family, MARCH1 and MARCH2 had the antiviral activity in a dose-dependent manner. The expression of these proteins in virus-producer cells decreased the efficiency of viral entry and indeed downregulated HIV-1 envelope glycoproteins from the cell surface, resulting in reduced incorporation of envelope glycoproteins into virions, exactly as observed in MARCH8 expression. Endogenous expression of MARCH1 and MARCH2 was enhanced in monocyte-derived macrophages by treatment with type I interferon.

Conclusions: As the antiviral MARCH family members, MARCH1 and MARCH2 join a growing list of host factors that inhibit HIV-1 infection.