

## Exposing alterations in the nuclear proteome in HIV-infected human monocyte-derived Macrophages

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It is known that mononuclear phagocytes (MP) contribute to human immunodeficiency virus 1 (HIV-1) persistence by acting as reservoirs capable of harboring latent virus and facilitating productive infection. Understanding the molecular mechanisms occurring at the nuclear level that are involved in promoting this viral persistence in the MP may provide new avenues for treatment. We hypothesize that systemic proteomic profiling of nuclear proteins from HIV-/+ MP, and specifically macrophages will provide new insights on the virus controls these cells. As an *in vitro* experimental model, we used human monocyte-derived macrophages (MDM) and the laboratory adapted HIV-1<sub>ADA</sub>. In this model, monocytes were differentiated into macrophages for 7 days and collected; or MDM were either maintained in culture or infected with HIV-1<sub>ADA</sub> at MOI 1 for an additional nine days (total of 17 days). After day 7 or 17, nuclei are harvested and cytosolic, soluble nuclear and insoluble nuclear proteins were separated. Proteins including nuclear transcription factors, which are largely located in the soluble nuclear protein fraction, were digested using trypsin. Peptides were then separated based on isoelectric point and analyzed by liquid chromatography-electrospray ionization coupled tandem mass spectrometry (LC/ESI-MS/MS). Spectra were searched using Proteome Discoverer v1.2 software and the SEQUEST algorithm. The total soluble nuclear protein recovered from 24 million uninfected MDM after 7 days of differentiation ranged between 21-38 µg. After day 17, the total nuclear proteins from same number of cells were collected from HIV- and HIV+ samples were 31 µg and 29 µg, respectively. This comprises a 6% loss of proteins due to infection. MS/MS analysis of 20 µg of soluble nuclear proteins from uninfected MDM resulted in the identification of 2090 unique proteins. Of those, 544 are specifically localized to the nucleus, 977 are known to have subcellular localization and the localization of another 569 has not yet been reported in databases. These 569 proteins can be classified based on their function and 20 are kinases or phosphatases, 8 are ion channels or transporters, another 34 are enzymes, 11 are peptidases, and the remaining 496 are classified as other. In conclusion, this project highlights a novel approach to understanding the molecular mechanisms of HIV-1 infection in MDM at the nuclear level.