

Role of cystatin B/STAT-1 interaction in HIV replication

Rivera-Rivera .L, Pérez-Laspiur .J, Colon .K and L. M. Meléndez

Department of Microbiology and NeuroAIDS Program, University of Puerto Rico School of Medicine, Puerto Rico

Cystatin B, a cysteine protease inhibitor, is correlated with increased HIV replication in monocyte-derived macrophages (MDM) as demonstrated by proteome and gene silencing with siRNA. Subsequently, the signaling mechanisms for cystatin B in HIV replication were related to its interaction with signal transducer and activator of transcription-1 (STAT-1). Whereas STAT-1 activates HIV-1 replication, high levels of tyrosine phosphorylated STAT-1 (STAT-1PY) has been associated with HIV-1 inhibitory activity. In MDM, high levels of cystatin B correlate with a reduction of STAT-1PY. The aim of this study is to elucidate the mechanism by which cystatin B contributes to HIV-1 replication by regulation of STAT-1PY. We used immunoprecipitation and LC-MS/MS to identify the proteins interacting with cystatin B in order to elucidate the relationship between cystatin B, STAT-1 phosphorylation and HIV replication in MDM. Cystatin B/STAT-1 interaction associated with the increase HIV replication in MDM is part of a multiprotein complex that includes other proteins. Cystatin B interacted with many different proteins in HIV infected cells including regulatory, glycolytic, redox, structural protein, transport, and signaling proteins. Among them we found the Major Vault Protein (MVP), coded by an IFN- γ responsive gene that interferes with IFN- γ activated JAK/STAT signals) and Pyruvate Kinase M2 isoform (PKM2) that has been reported as an up-regulated protein associated with the effects of cocaine on the enhancement of HIV-1 replication. In situ Proximity Ligation Assay (Duolink) was used to determine how the HIV infection alters the cystatin B/STAT-1 interaction and the STAT-1PY in uninfected and HIV-infected MDM at 12 days post-infection. Cystatin B and STAT-1 interact directly and this interaction increases after HIV infection. However, the small interaction between STAT-1/STAT1PY observed in control uninfected was decreased after HIV infection. To test that cystatin B inhibits the IFN- β response, we performed luciferase reporter gene assays in Vero cells, which are IFN deficient. We demonstrated that cystatin B inhibited the IFN- β response by preventing STAT-1 translocation to the nucleus and decreasing levels of STAT-1PY. Taking together, we concluded that the cystatin B/STAT-1 interaction inhibits the signaling pathway mediated by IFN- α by regulating STAT-1PY and/or its nuclear translocation. This action would prevent the expression of IFN-dependent antiviral genes and promote HIV replication mediated by LTR and IRF-1. This work was supported in part by NIH grants F32MH094210-01A1, R01-MH08316-01, RCMI-NCRR-G12RR03051, SNRP-NINDS-1-U54NS431.

linda.rivera2@upr.edu