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Genome-wide transcriptional profiling reveals that HIV-1 Vpr differentially regulates interferon-stimulated genes in human monocyte-derived dendritic cells

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ABSTRACT

Dendritic cells (DCs) are potent antigen-presenting cells (APCs) that directly link the innate and adaptive immune responses. HIV-1 infection of DCs leads to a diverse array of changes in gene expression and play a major role in dissemination of the virus into T-cells. Although HIV-1 Vpr is a pleiotropic protein involved in HIV-1 replication and pathogenesis, its exact role in APCs such as DCs remains elusive. In this study, utilizing a microarray-based systemic biology approach, we found that HIV-1 Vpr differentially regulates (fold change >2.0) more than 200 genes, primarily those involved in the immune response and innate immune response including type I interferon signaling pathway. The differential expression profiles of select genes involved in innate immune responses (interferon-stimulated genes [ISGs]), including *MX1*, *MX2*, *ISG15*, *ISG20*, *IFIT1*, *IFIT2*, *IFIT3*, *IFI27*, *IFI44L*, and *TNFSF10*, were validated by real-time quantitative PCR; the results were consistent with the microarray data. Taken together, our findings are the first to demonstrate that HIV-1 Vpr induces ISGs and activates the type I IFN signaling pathway in human DCs, and provide insights into the role of Vpr in HIV-1 pathogenesis.

1. Introduction

Dendritic cells (DCs), the most potent antigen-presenting cells (APCs) in the immune system, act as a link between the innate and adaptive immunity (Banchereau and Steinman, 1998). DCs are among the first cells to encounter human immunodeficiency virus type 1 (HIV-1) through mucosal surfaces, which facilitate the virus' access to $CD4^+$ T cells and its subsequent systemic dissemination (Banchereau and Palucka, 2005). HIV-1 exploits a diverse array of host genes in order to replicate and cause disease, which is mediated by a complex network of host–virus interactions (Wu and KewalRamani, 2006).

HIV-1 Vpr is a 14 kDa pleiotropic protein that plays multiple biological functions: it promotes nuclear import of the preintegration complex (Aida and Matsuda, 2009; Heinzinger et al., 1994; Kamata et al., 2005; Nitahara-Kasahara et al., 2007; Popov et al., 1998; Takeda et al., 2011), HIV-1 long terminal repeat-driven transcription in infected cells (Kino et al., 2002), envelope protein expression and virion production in macrophages (Mashiba et al., 2014), regulation of splicing (Hashizume et al., 2007; Kuramitsu et al., 2005), induction of apoptosis (Murakami and Aida, 2014; Nishizawa et al., 2000), and cell cycle arrest at G2/M phase (Laguette et al., 2014; Murakami and Aida, 2014). Vpr exerts significant effects on cellular proliferation and differentiation, modulates cytokine production, and suppresses host cell-mediated nuclear factor-κB (NF-κB) activation (Ayyavoo et al., 1997; de Noronha et al., 2001; Jowett et al., 1995; Levy et al., 1993; Varin et al., 2005).

Dysregulation of DCs occurs during the course of HIV-1 disease (Sabado et al., 2010). Moreover, the early events in HIV-1–host interactions are likely to make critical contributions to disease progression (Neil and Bieniasz, 2009; Wu and KewalRamani, 2006). Vpr protein, which is enclosed within HIV-1 virions and is capable of causing cell cycle arrest in $CD4^+$ T cells (Poon et al., 1998), is one of the HIV-1 proteins encountered by host cells during the early stage of infection (Cohen et al., 1990; Poon et al., 1998). Vpr impedes the maturation of DCs (Muthumani et al., 2005) and is considered to be an essential component for HIV-1 replication in non-dividing human cells (Connor et al., 1995; Nitahara-Kasahara

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et al., 2007; Poon et al., 1998); however, its exact role in dendritic cells remains elusive. In this study, we used microarrays to identify host cell genes that are differentially regulated by HIV-1 Vpr in monocyte-derived dendritic cells (MDDCs), which are used as a model for myeloid DCs (Zhou and Tedder, 1996). We found that HIV-1 Vpr up-regulates interferon (IFN)-stimulated genes (ISGs) and activates the type I IFN signaling pathway in human DCs. This is the first report to show that HIV-1 Vpr induces ISGs in human DCs. Our findings expand our understanding of HIV-1 replication and pathogenesis in human DCs.

2. Materials and methods

2.1. Cell culture and preparation of human MDDCs

HeLa and HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% fetal bovine serum (Sigma) and penicillin/streptomycin. HeLa cells were used for cell cycle analysis, whereas HEK293 cells were used for the generation of recombinant adenoviruses expressing ZsGreen1 and HIV-1 Vpr proteins, essentially as described in our recent report (Zahoor et al., 2014).

PBMCs were obtained using a standard Ficoll gradient from heparinized blood extracted from two healthy individuals. $CD14^+$ cells were isolated by positive selection with anti-human $CD14^+$ magnetic beads (Miltenyi); the purity of the resultant cells was at least 95% (data not shown). Primary immature monocyte dendritic cells (iMDDCs) were generated by culturing $CD14^+$ cells with GM-CSF and IL-4 (PeproTech) (Bender et al., 1996). At 7 day after plating, cells exhibited the typical dendritic cell morphology, and their differentiation status was confirmed by detection of iMDDC surface markers ($CD86^+$, $CD83^-$, $CD11c^{low}$, $CD1a^{high}$, $CD14^-$, and $HLA\text{-}DR^{high}$).

2.2. Antibodies

HIV-1 Vpr mouse monoclonal antibody (mAb) #3 was produced by immunization with the peptide N-CQAPEDQGPQREPYN-C, corresponding to amino acids 3–16 of Vpr, as described previously (Zahoor et al., 2014). ZsGreen1 rabbit polyclonal antibody (#632474) was purchased from Clontech Laboratories. Fluorescein isothiocyanate-conjugated mAbs directed against the human surface markers CD86, CD83, CD11c, CD1a, CD14, and HLA-DR were obtained from Miltenyi Biotec and used at the supplier's recommended concentrations. β-actin (#1978) mAb and horseradish peroxidase (HRP)-labeled goat anti-mouse or goat anti-rabbit secondary antibodies were purchased from Sigma. Ethical approval for this study was granted by the RIKEN Ethics Committee [Certificate No. Wako 21-2(3)].

2.3. Flow cytometry analysis

After staining, MDDCs were analyzed on a FACSCalibur™ flow cytometer (BD Japan) and the data were analyzed using FCS Express (Ver. 3; De Novo Software).

2.4. Western blotting analysis

Mock or virus-infected MDDCs were washed with phosphate buffered saline (PBS) and then lysed with CelLytic™ MT Cell Lysis reagent (Sigma) supplemented with protease inhibitor cocktail (Roche Diagnostics) according to the manufacturer's instructions. Protein concentrations were determined using the BCA Protein Assay kit (Pierce) using bovine serum albumin as a standard. Proteins were separated by sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis and transferred to polyvinyl difluoride (PVDF; Millipore Corp.) membranes. PVDF membranes were probed with the primary antibodies listed above, followed by incubation with HRP-conjugated secondary antibody (Sigma). Signals were detected by enhanced chemiluminescence (GE Healthcare).

2.5. Generation of recombinant adenoviruses

Recombinant adenoviruses expressing HIV-1 Vpr or ZsGreen1 proteins were constructed using the Adeno-X™ expression system (Clontech Laboratories) as described previously (Zahoor et al., 2014).

2.6. RNA extraction

MDDCs were transduced with Ad-Vpr or Ad-Zs at an MOI of 100. The cells were harvested for RNA extraction at 48 h post-transduction. MDDCs were washed three times with ice-cold PBS, and total RNA was extracted using the RNeasy Mini kit with DNase digestion (Qiagen). RNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher) and stored at −80 °C. For microarray analysis, quality of RNA was determined using an Agilent Bioanalyzer (Agilent Technologies).

2.7. Microarray and data analysis

RNA samples were analyzed using the GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix). Microarray hybridization and fluorescence detection were performed as described in the Affymetrix GeneChip Expression Analysis Technical Manual. The .CEL data files generated by the Affymetrix microarray hybridization platform were analyzed using the GeneSpring GX software, ver. 12.0 (Agilent Technologies). Probe-level analysis was performed using the Robust Multi-array Average (RMA) algorithm. Microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO) and assigned the GEO Series accession number GSE61171. Fold changes in gene expression were determined, and hierarchical clustering and gene ontology analyses were performed.

2.8. Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis of differentially expressed genes

Total RNA was prepared using the RNeasy Mini kit, as described above. qRT-PCR was performed using specific primers, as described (Zahoor et al., 2014), One-Step SYBR Green PCR mix (Takara) and a Prism 7500 sequence detection system (Applied Biosystems). Samples were run in triplicate, and all data were normalized to *GAPDH* mRNA expression as an internal control.

3. Results

3.1. Expression of Vpr and ZsGreen1 in human MDDCs

To better understand the role of HIV-1 Vpr in MDDCs, we performed a genome-wide global transcriptome analysis of MDDCs infected with a recombinant adenovirus expressing HIV-1 Vpr. To this end, we generated MDDCs, as shown in Fig. 1A. First, $CD14^+$ cells were isolated from peripheral blood mononuclear cells (PBMCs) obtained by leukophoresis from two healthy donors, as described previously (Zahoor et al., 2014). Next, adherent $CD14^+$ monocytes were cultured for 7 days in the presence of IL-4 and GM-CSF, as described (Bender et al., 1996; Zhou and Tedder, 1996). The differentiation stage of the cells was determined by monitoring the expression of cell-surface markers using flow cytometry (Fig. 1A and 1B). On day 7, the cells exhibited phenotypic and morphologic characteristics of immature DCs: they expressed $CD86^+$, very high levels of $CD1a^+$ and $HLA\text{-}DR^+$, and low levels of $CD11c^+$, and they