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Expression Profiles and Pathway Analysis in HEK 293 T Cells Overexpressing HIV-1 Tat and Nucleocapsid Using cDNA Microarray

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Abstract Human immunodeficiency virus type 1 (HIV-1) infections are responsible for a substantial number of deaths annually and represent a significant threat to public health. According to the latest study, the Tat (Transactivator of transcription) protein is essential in transcription and replication of viral genes, and is among the early expression genes involved in the life cycle of HIV. The virion NC (nucleocapsid) plays an important role in early mRNA expression and contributes to the rapid viral replication that occurs during HIV-1 infection. Therefore, we attempted to elucidate the relationship between the Tat protein and nucleocapsid protein. In a comparison of two independently prepared and hybridized samples, flag NC overexpressed HEK 293T cells and pTat overexpressed HEK 293T cells, and hybridization showed the differences in expression in each case. Among the microarray results confirmed with real-time reverse transcriptase assay, twelve genes were identified to be involved according to their gene expression profiles. Of approximately 8,208 human genes that were analyzed, we monitored candidate genes that might have been related to NC and Tat genes from gene expression profiles. Additionally, the pathways could be viewed and analyzed through the use of PathwayStudio software. The pathways from the gene list were built and paths were found among the molecules/cell objects/processes by the curation method.

Key words: Profiles, pathway, HIV-1, NC, Tat

AIDS stands for acquired immunodeficiency syndrome, a pattern of devastating infections caused by human immunodeficiency virus type 1, which attacks and destroys certain white blood cells that are essential to the immune system. Investigation of the replication mechanism of HIV or gene and protein levels is very important to the development of treatments and cures to conquer HIV. The cell cycle of HIV-1 continues through gene regulation of the host protein. However, the gene regulation mechanism of HIV-1 has not yet been properly elucidated.

Tat protein acts as a main modulator of HIV as a gene that is revealed during a relatively early period of the life cycle of HIV-1. The HIV-1 genome consists of about 9,000 nucleotides, with long-term repeats (LTR) at both ends. The genome consists of three genes: *gag*, *pol*, and *env*. The LTR acts as a host of DNA integration, and the complete HIV genome is transcripted as a primer or regulator. The *pol* gene encodes the viral enzymes required for replication, and the *env* gene encodes glycoproteins that become part of the viral envelope. The *gag* gene is translated into a polyprotein that is sufficient to mediate the formation of virion-like particles (VLP) in the absence of other viral proteins [1]. The Tat protein appears to enhance posttranscriptional events such as transport of new mRNAs from the nucleus. Whereas the Tat protein stimulates expression of the entire viral genome, the Rev protein is a transactivator necessary only for the synthesis of the major viral structural polyproteins. Firstly, Tat (Trans Activating Factor), acts as a transcription transactivator, binding to the TAR sequence that is situated in the cDNA of the virus and is an important protein that acts during late gene expression of the virus. In addition to its nuclear localization and function, Tat is also released from infected cells extracellularly and is endocytosed by neighboring cells, where it translocates to the nucleus in an active form [6]. Since its discovery, much of the mainstream human immunodeficiency virus type 1 (HIV-1) Tat research has

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focused on its ability to activate the HIV-1 LTR [5]. Tat protein accumulates in the nuclei of HIV-infected cells, but can also act as a pleiotropic exogenous factor because of its ability to induce various biological effects in different cell types [3].

Nucleocapsid (NC) is a structural protein that acts as packaging for viral genomic RNA. NC protein is encoded by the 3' region of *gag* and is well conserved among retroviruses. Both NCp15 and NCp7 proteins have been detected in HIV-1 virions [10]. NCp7 is a highly basic protein that contains two zinc fingers of the form Cys-X2-cys-X4-His-X4-Cys (CCHC) flanked by regions rich in basic residues [24].

The splicing silencer exists if one examines the structures of the exon and intron of this *tat* gene through the full-length coding sequence RNA. Because of this, it has been assumed that there must be a mechanism by which the genome can overcome splicing repression. Recently, the cell protein of concern became known as hnRNP A1 protein. Therefore, in this study, we attempted to elucidate the mechanism influencing the direct or indirect interaction of nucleocapsid in the activation and expression of Tat. This may offer new information regarding the two protein control mechanisms of early gene expression of HIV and their interaction. Whole-genome expression profiling exemplified by the development of DNA microarrays represents a major advance in genome-wide functional analysis [22, 20, 12, 18, 14].

Therefore, the aims of the present study were to monitor the gene expression aspect in Tat and nucleocapsid protein expression cells and to search their relativity in gene level by taking advantage of a microarray technology to determine the relation with interrelationship and cellular factor between Tat and nucleocapsid protein that is the main control genetic factor belonging to early among life cycle of HIV-1.

MATERIALS AND METHODS

Cell Culture and Transfection Assay

The HEK 293T human embryonic kidney cell line was culture in Dulbecco's modified Eagle's medium (JBI) supplemented with 10% (v/v) heat-inactivated (56°C, 30 min) Fetal Bovine Serum (JBI) and 1% penicillin-streptomycin, and was maintained at 37°C in a CO_2 environment. For transient transfection, the pCMV(-HA) flag NC and pTat were isolated from a 50-ml culture using a Plasmid Midi Kit (Qiagen, Chatworth, CA, U.S.A.).

Approximately 3×10^5 HEK 293T cells were plated in a T75 flask 24 h before transfection, by which time they reached 90–95% confluency. For transient transfection, separate pCMV(-HA) flag NC plasmid DNA and pTat plasmid DNA were mixed with Lipofectamine 2000. Transient transfections were performed by following the protocol of the manufacturer. For co-transfection experiments, pCMV(-HA) flag NC plasmid DNA and pTat plasmid DNA were mixed with Lipofectamine 2000. The DNA-Lipofectamine 2000 complex was added to each well containing HEK 293T cells and medium. After 4–6 h at 37°C, the media containing the transfection mix was removed and 10 ml of growth medium (DMEM, 10%FBS) was added. The cells were washed 24 h after transfection, and fresh media was added to the cells. At 48 h post-transfection, whole cells were harvested.

RNA Isolation

Approximately 3×10^5 cells were plated in a T75 flask. When cells reached confluency, they were trypsinized and washed with PBS, and were then centrifuged at 1,500 rpm for 5 min. Freshly isolated cells from each separate culture were lyzed in easy-blue solution (Intron). The RNA was extracted from the cell lysate by the addition of 0.2 vol. of chloroform. RNA was precipitated with isopropanol in the aqueous phase and washed in 70% ethanol. The RNA pellet was dissolved in diethylene-pyrocarbonate (DEPC)-treated water. The total RNA was assessed using a spectrophotometer (Nanodrop technologies) within a 260/280 nm OD ratio of 1.8–2.0.

cDNA Microarray Hybridization

After washing normal HEK 293T cells, total RNA from pCMV(-HA) flag NC-transfected HEK 293T cells, pTat-transfected HEK 293T cells, pCMV(-HA) flag NC, and pTat co-transfected HEK 293T cells with PBS was prepared using easy-blue solution according to the instructions of the manufacturer. Using the 3DNA 50 Expression array detection kit (Genisphere, Newark, NJ, U.S.A.), 10–20 μg of total RNA was reverse-transcribed, labeled with Cy3 and/or Cy5, and hybridized to the microarray according to the instructions of the manufacturer. Slides were cover-slipped and the mixture hybridized at 65°C for 16 h in a sealed chamber (Telechem, U.S.A.). The slides were then washed once in 1× SSC, 0.1% SDS solution at 65°C for 10 min, and twice in 0.2× SSC at room temperature for 10 min. Slides were dried by centrifugation at low speed prior to scanning.

Scanning and Data Analysis

Images were obtained by scanning the arrays in an arrayWoRx scanner (Applied Precision Inc. Northwest Issaquah, U.S.A.). Signal intensities for Cy3- and Cy5-labeled probes were extracted with the ImaGene software package, version 5.0 (BioDiscovery, Inc., Marina Del Rey, CA, U.S.A.) using default settings and auto image segmentation. Mean and median intensities for signal and background as well as quality characteristics ("empty" or "poor") of the spots were obtained at this time. The threshold for empty spots was achieved by raising the

threshold to a point at which all blank spots were flagged. The data were then analyzed by hierarchical clustering (GeneSight software package, version3.5, BioDiscovery).

RT-PCR

Five μg of total RNA was reverse-transcribed in a 20-μl reaction volume containing oligonucleotide dTs(dT18) and 10 mM dNTP (Amersham Pharmacia Biotech Inc., Piscataway, NJ, U.S.A.) using M-MLV reverse transcriptase (Promega). The mixture was incubated at 70°C for 5 min and placed on ice for 1 min. RNase inhibitor and 5× reaction buffer were then added to the mixture. The solution was incubated at 42°C for 1 h, and heated at 70°C for 10 min. PCR was performed in a 50 μl reaction mixture containing 10 pmol dNTP (Bioneer, Republic of Korea) and 10× reaction buffer. This solution was incubated 94°C for 5 min, 33 cycles of 94°C for 30 sec, 52–58°C for 45 sec, and 72°C for 1 min. The final extension time was 7 min.

PathwayStudio Analysis

PathwayStudio builds pathways using various methods. This software has a built-in resource called ResNet, which is a database for searching molecular interactions through natural language processing of scientific abstracts contained in the PubMed database. From ResNet, we were able to add our interesting gene product onto a new pathway diagram and build a new pathway based on well-known interactions referred to in the relevant literature.

Pathway Reconstruction Algorithms

The algorithm in PathwayStudio constructs a pathway based on the interaction between proteins. Initially, it starts with a ligand-receptor pair and finds all proteins related to the receptor and ligand in the ResNet database. It also connects all found downstream proteins using physical interactions such as binding or protein modification, and removes unconnected targets. In addition, a second algorithm

Table 1. Flag NC, upregulated (12 genes); and Flag NC, downregulated (8 genes).

Accession no.	Up-regulated genes and downregulated genes	Fold charge
	Gene about the HIV-1	
NM_000584	Interleukin 8 (IL8)	7.411
NM_000073	CD3G antigen, gamma polypeptide (TiT3 complex) (CD3G)	3.881
NM_000212	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) (ITGB3)	3.077
	Regulation of expression	
NM_001419	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R) (ELAVL1)	7.966
NM_001832	Colipase, pancreatic (CLPS)	4.587
	ATP binding	
NM_000119	Erythrocyte membrane protein band 4.2 (EPB42)	7.586
	Cell proliferation	
NM_003896	Sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase; GM3 synthase) (ST3GAL5)	5.832
NM_000948	Prolactin (PRL)	5.201
	Apoptosis	
NM_005894	CD5 antigen-like (scavenger receptor cysteine-rich family) (CD5L)	4.149
	Fatty acid binding	
NM_001442	Fatty acid binding protein 4, adipocyte (FABP4)	4.406
	Unknown	
Hs.75939	Uridine monophosphate kinase (UMPK)	7.112
Hs.26937	Brain and nasopharyngeal carcinoma susceptibility protein (NSG-X)	4.788
	Gene about the HIV-1	
NM_000311	prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) (PRNP)	0.179
NM_003542	H4 histone family, member G (HIST1H4C)	0.230
NM_003544	H4 histone family, member I (HIST1H4B)	0.246
	RNA splicing	
NM_020230	Peter pan (Drosophila) homolog (PPAN)	0.167
	Initiation factor	
NM_002453	Mitochondrial translational initiation factor 2 (MTIF2)	0.165
NM_004095	Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1)	0.386
	Apoptosis	
NM_001191	BCL2-like 1 (BCL2L1)	0.266
NM_001767	CD2 antigen (p50), sheep red blood cell receptor (CD2)	0.269

assigns weights to all connections in ResNet by scoring the reference count and the number of similar relationships for paralogous proteins. It then determines the optimal path in the weighted graph between the receptor and the downstream transcription factor using physical interactions in ResNet. This algorithm considers only transcription factors that are regulated by the receptor in the ResNet database.

RESULTS

Transfection in the HEK 293T Cells and Application of cDNA Microarray

The nucleocapsid protein of the HIV-1 was found to be of low efficiency; therefore, we used HEK 293T cells with high efficiency. After transfection for 48 h, we performed semiquantitative RT-PCR for detecting the expression of flag NC. GAPDH mRNA amplification was used as a constitutive control. On the basis of this data, further cDNA microarrays were performed.

The gene expression profiles of the flag nucleocapsid protein in HEK 293T cells were compared to that of the control HEK 293T cells using 8208 human cDNA microarrays. All data were obtained by normalization using analysis software, Imagene and Genesight. Hybridization was repeated three times and integrated for statistical meaning.

A histogram was created and regions were selected if their charges were greater than 4.8-fold or under 0.5-fold that of the control. Genes that were upregulated in flag NC-overexpressing HEK 293T cells participated in cell adhesion (PCDH7), proliferation (SIAT9 and PRL), and apoptosis (CD5L). Among these genes, IL8, CD3G, and ITGB3 are genes that function in the HIV-1 genome. Genes downregulated in flag nucleocapsid-overexpressing

Table 2. pTat, upregulated (9 genes); and pTat, downregulated (13 genes).

Accession no.	Upregulated genes and downregulated genes	Fold charge
	Gene about the HIV-1	
NM_000734	CD3Z antigen, zeta polypeptide (TiT3 complex) (CD247)	1.855
	A3 adenosine receptor	
NM_000677	Adenosine A3 receptor (ADORA3), mRNA (ADORA3)	2.305
	Calcium ion binding	
NM_000250	Myeloperoxidase (MPO)	1.887
	Cell adhesion	
NM_001337	Chemokine (C-X3-C) receptor 1 (CX3CR1)	1.839
	Transcription	
NM_004634	Bromodomain and PHD finger-containing, 1 (BRPF1)	1.839
	Integral to membrane	
NM_006894	Flavin containing monooxygenase 3 (FMO3)	3.454
	Cell proliferation	
NM_001772	CD33 antigen (gp67) (CD33)	1.725
NM_001316	Chromosome segregation 1 (yeast homolog)-like (CSE1L)	1.649
	Transferase activity	
NM_000561	Glutathione S-transferase M1 (GSTM1)	1.893
	Gene about the HIV-1	
NM_000041	Apolipoprotein E (APOE)	0.334
NM_005646	TAR (HIV) RNA-binding protein 1 (TARBP1)	0.545
NM_000484	Amyloid beta (A4) precursor protein (protease nexin-II, Alzheimer disease) (APP)	0.562
NM_002265	Karyopherin (importin) beta 1 (KPNB1)	0.597
	Zinc ion binding	
NM_007212	Ring finger protein 2 (RNF2)	0.310
NM_001644	Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 1 (APOBEC1)	0.553
NM_003110	Sp2 transcription factor (SP2)	0.567
NM_002196	Insulinoma-associated 1 (INSM1)	0.584
	Transcription	
NM_001265	Caudal-type homeobox transcription factor 2 (CDX2)	0.503
NM_004688	N-myc (and STAT) interactor (NMI)	0.508
	Integral to membrane	
NM_001057	Tachykinin receptor 2 (TACR2)	0.387
NM_000232	Sarcoglycan, beta (43kD dystrophin-associated glycoprotein) (SGCB)	0.559
NM_007000	Uroplakin 1A (UPK1A)	0.568

HEK 293T cells included some involved in initiation factor (EIF4EBP1), RNA splicing (PPAN), and HIV-1 functions (PRNP, HIST1H4C, and HIST1H4B) (Table 1). Additionally, 11 upregulated genes that have cell adhesion molecule function were identified in flag NC-overexpressing HEK 293T cells (data not shown).

HEK 293T cells were used for pTat gene transfection. After 48 h transfection, semiquantitative RT-PCR was performed for detecting the expression of the pTat gene. pTat as a flag NC was used for the experiments, which were performed in triplicate. The sections of the histogram showed greater than 1.8-fold and less than 0.6-fold charges compared with control were selected for further analysis. Genes upregulated in pTat-overexpressing HEK 293T cells included A3 adenosine receptor and participated in the immune response. We can know that genes (CD33, CSE1L) related to cell proliferation were upregulated in the flag NC. The downregulated genes in pTat-overexpressing HEK 293T cells are included in zinc ion binding (RNF2, APOBEC1, SP2, INSM1) and HIV functions (APOE, TARBP1, APP, KPNB1) (Table 2).

Verification of Candidate Genes by Semiquantitative RT-PCR

To validate the data obtained by microarray analysis, differentially expressed transcripts representing upregulated or downregulated genes were analyzed by reverse-transcription PCR (RT-PCR). Genes found to be highly upregulated or downregulated compared with a reference sample are often examined more closely using these techniques.

As shown in Figs. 1 and 2, we selected eight and four genes identified by the cDNA microarray to undergo RT-PCR analysis using RNA isolated from flag NC- and pTat-transfected HEK 293T cells, respectively. A parallel RT-PCR performed with the GAPDH primers showed that the expression of this housekeeping gene was equal in the transfected and control cells. We found the same direction of change in reverse transcriptase-PCR as with the microarray experiments in all twelve cases.

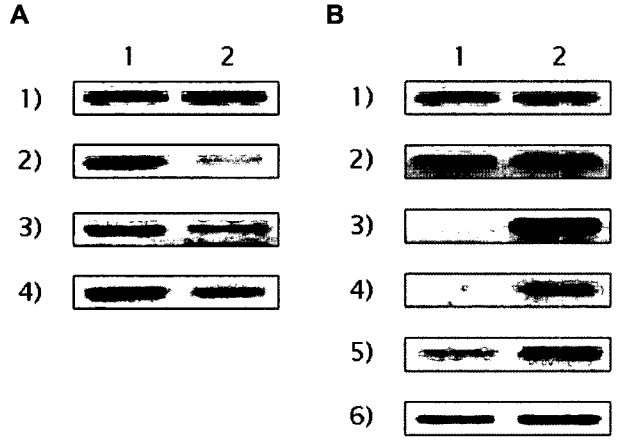

Fig. 1. Verification of genes selected from pNC-transfected HEK 293T cells by semiquantitative RT-PCR.
A. Upregulated genes in flag NC-transfected HEK 293T cells. Lane 1, pNC-transfected HEK 293T cells. Lane 2, HEK 293T cells. 1) GAPDH, 2) IL-8. 3) ELAV, 4) SIAT7. **B.** Downregulated genes in flag NC-transfected HEK 293T cells. Lane 1, pNC-transfected HEK 293T cells. Lane 2, HEK 293T cells. 1) GAPDH, 2) PRL. 3) HIST1H4C, 4) HIST1H4B, 5) BCL2L1, 6) EIF4EBP1.

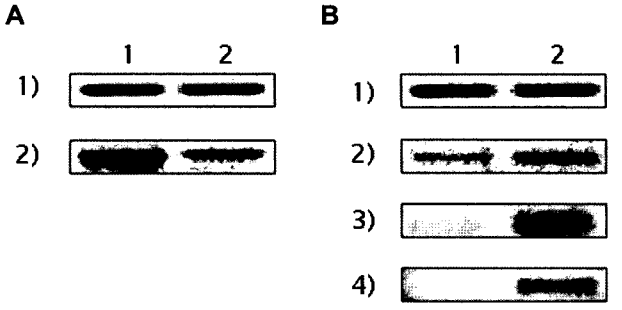

Fig. 2. Verification of genes selected from pTat-transfected HEK 293T cells by semiquantitative RT-PCR.
A. Upregulated genes in flag Tat-transfected HEK 293T cells. Lane 1, pTat-transfected HEK 293T cells. Lane 2, HEK 293T cells. 1) GAPDH, 2) ADORA. **B.** Downregulated genes in flag Tat-transfected HEK 293T cells. Lane 1, pTat-transfected HEK 293T cells. Lane 2, HEK 293T cells. 1) GAPDH, 2) APOE. 3) TARBP1, 4) APP.

Gene Expression Profiles in Clusters

In order to comprehensively analyze the change of gene expression, we applied a hierarchical clustering format to explore the gene expression data derived from cDNA microarray analysis. Fig. 3 shows the cluster images of different gene expression profiles. Each row represents a

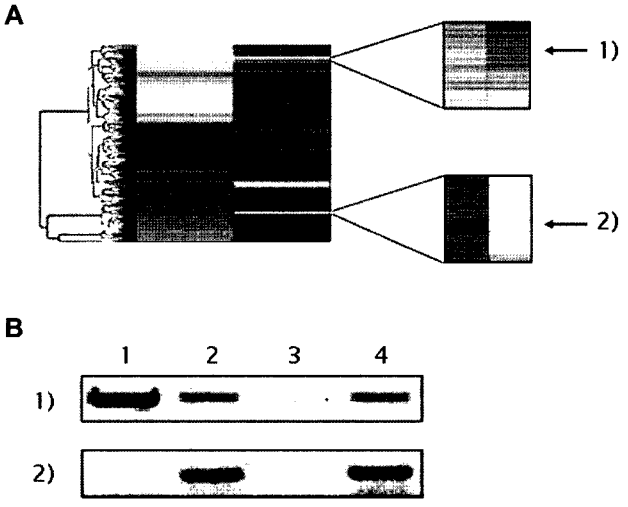

Fig. 3. Gene expression profiles in hierarchical clusters.
A. Hierarchical cluster. 1) COLQ, 2) USP20. **B.** Verification of genes selected after hierarchical clustering by semiquantitative RT-PCR. Lane 1, NC-transfected HEK 293T cells. Lane 2, HEK 293T cells. Lane 3, pTat-transfected HEK 293T cells, Lane 4, HEK 293T cells. 1) COLQ, 2) USP20.

separate cDNA clone on the microarray, and each column indicates a separate mRNA sample. These ratios were a measure of relative gene expression in each experimental sample and were depicted according to the color scale shown at the upper left. Genes that are upregulated appear in red, and those that are downregulated appear in green. This method of clustering group genes by reordering the expression matrix allowed patterns to be visualized easily.

After gene clustering, we selected genes of two types. One is the downregulation of the NC gene and Tat gene, the other is the up- and downregulation of the NC gene and pTat gene. USP20 is the gene that is upregulated to the flag NC transfected in the HEK 293T cells. At the same time, it is also the gene that is downregulated to pTat transfected in HEK 293T cells. COLQ; flag NC downregulated and pTat downregulated in HEK 293T cells (Fig. 3).

Building and Visualizing Pathways with PathwayStudio

PathwayStudio built several pathways from gene lists given by the user and found paths among molecules/cell objects/processes. We described the methodology for automatic curation of Biological Association Networks derived by a natural language processing technology. The curated data are used for automatic pathway reconstruction. The algorithm for the reconstruction of signaling pathways is also described and validated by comparison with manually curated pathways and gene-specific expression profiles. Based on the expression profiles of NC-overexpressing HEK 293T cells, the possible pathway was obtained. In Fig. 4, each color and symbol in the interaction between proteins is informative in terms of particular relationships such as HIV function, regulation of expression, cell proliferation, or molecular transport.

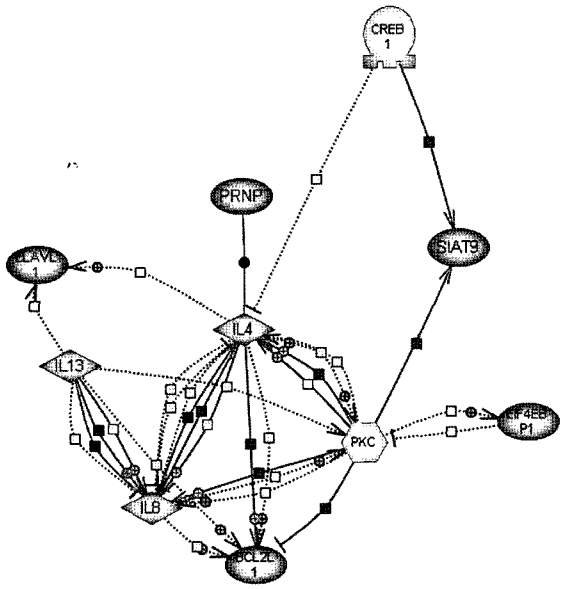

Fig. 4. Manually built NC pathway of the genes from "flag NC and pTat upregulated genes."
Graph legend: IL-8 is shown as a red rhombus, and ELAV, SIAT9, PRL, BCL2L1, and EIF4EBP1 red sickle shapes.

DISCUSSION

Acquired immunodeficiency syndrome (AIDS), the disease induced by HIV-1, is a complex process that involves both multiple viral and host determinants. The development of AIDS can be viewed as occurring in three phases: a brief primary infection, followed by a long asymptomatic period, finally resulting in disease manifestation and death [21].

The NC (nucleocapsid) protein, which contains two zinc-finger motifs thought to be important for packaging of the viral genomic RNA, binds to genomic RNA [11]. Tat protein is a transcriptional transactivator with a zinc-finger structure. Tat protein binds to the specific sequences of TAR (Transactivation Response Element), located in the R region of LTR5 [3]. Therefore, the NC protein is a component that is very similar to Tat, but its role is not clear. To clarify the roles of NC and Tat in HEK 293T cells, we used a human cDNA microarray containing 8,208 human genes to identify the relation between overexpression of the nucleocapsid and Tat proteins. Arrays that have been used to monitor differences in expression profiles following global changes in cellular physiology demonstrate a large number of differences in expressed transcripts between samples. Modulation of gene expression was quantitated by calculating the ratio of the intensity of the normalized hybridization signal. Data preparation consisted of the following steps: using mean signal and background intensities, background correction was performed by subtracting the local background value from each spot; spots that were flagged as empty or poor were omitted from the analysis; the intensity ratio of the two channels was calculated. The replicate experiments were combined, and the mean between the intensity ratios for the series of experiments was calculated. Arrays were then clustered using the hierarchical clustering algorithm.

Through a microarray approach, the HIV-1 NC is able to regulate many cellular genes that are involved in cell signaling, translation, and, ultimately, controlling the proliferative and differentiation signals of the host (Table 1). We confirmed the IL-8, ELAV, SIAT9, PRNP, EIF4EBP1, HIST1H4C, HIST1H4B, and BCL2L1 genes again using semiquantitative RT-PCR among the upregulated and downregulated genes. IL-8 has been demonstrated to attract neutrophils and T cells, stimulate monocyte adherence, and mediate angiogenesis by interacting with the CXC chemokine receptors, CXCR1 and CXCR2 [2, 9, 13, 16, 25]. Exposure of MDM (monocyte-derived macrophages) to HIV-1 leads to increased IL-8 production, an effect mediated by Tat and the inflammatory cytokine tumor necrosis factor

alpha, as well as by gp120 [15]. Among the downregulated genes, EIF4EBP1 is one of a variety of translation initiation factors that are modified upon induction of apoptosis [4], and eIF4E appears to be important in modulating programmed cell death [23, 19]. After flag NC transfection in HEK 293T cells, flag NC-overexpressing HEK 293T cells and control HEK 293T cells showed definite differences in adhesion in the period of observation. We could observe that the expression patterns of other molecules, such as integrin, increased adhesion molecule function. The SIAT9 protein is known to participate in the induction of cell differentiation, modulation of cell proliferation, maintenance of fibroblast morphology, signal transduction, and integrin-mediated cell adhesion.

Using the latest repository, it was found that Tat overexpressed in epithelial cells increases adhesion function via the NF-κB pathway. Additionally, according to a previous study, Tat and integrin protein interacted with each other for the immobilization of Tat. Based on this finding, it appears that the relationship between the NC protein and Tat is important. Recent reports have suggested that the Tat protein might represent a promising target protein for the control of HIV infection owing to its critical role in virus replication and disease progression [8]. Among the upregulated genes, adenosine A3 has been proposed to be a "metabolic" switch that may sense and direct immune and inflammatory responses [7]. Adenosine A3 can influence the functions of monocytes, macrophages, neutrophils, T cells, and dendritic cells, suggesting that adenosine may play a key role in regulating inflammatory and immune responses (Table 2). Additionally, among the downregulated genes, we understand that the TAR gene is functionally related to NC. Nucleocapsid variants with mutations in their zinc-finger domains have dramatically altered TAR RNA binding interactions relative to wild-type NC. A specific zinc-finger architecture is required for optimal TAR RNA binding, and helps to explain the requirements for the zinc-finger motifs of NC in its role as a nucleic acid chaperone in minus-strand transfer [17]. We can assume that Tat and NC are in affinity through this (Tables 1 and 2).

We clustered the genes that were up- or downregulated in each test. Through clustering, we were able to determine a number of gene profiles and focused on the case of NC downregulated, Tat downregulated, NC upregulated genes, and Tat downregulated genes among them. Genes such as COLQ and USP20 belong to these groups. We did various experiments, including gene profile analysis, in order to understand the relationship between nucleocapsid and Tat more clearly. To find more specific functions, we plan to construct flag NC stable cell lines, pTat stable cell lines, and co-transfection stable cell lines. This step will allow us to provide information regarding the detailed mechanism of protein levels.

Studying the pathway analysis of the role of NC protein and pTat protein in HIV functions is one of the keys to understanding their interactions during virus packaging. Exploration of further candidate molecules involved in this pathway and demonstration of their precise roles will provide a more comprehensive view of the mechanism implicated in the regulation of the virus packaging system.

Acknowledgments

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