FEBS Letters 462 (1999) 182–186 FEBS 22959

Serial analysis of gene expression in HIV-1-infected T cell lines

Akihide Ryo[a], Youichi Suzuki[a], Kouji Ichiyama[a], Toru Wakatsuki[b], Nobuo Kondoh[b], Akiyuki Hada[b], Mikio Yamamoto[b], Naoki Yamamoto[a,*]

[a] *Departments of Microbiology and Molecular Virology, School of Medicine, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8519, Japan*

[b] *Department of Biochemistry II, National Defense Medical College, 3-23, Namiki, Tokorozawa, Saitama 359-0042, Japan*

Received 28 September 1999; received in revised form 2 November 1999

Abstract The gene expression profile of the HIV-1 infection state was analyzed in the human T cell line MOLT-4. Using the serial analysis of gene expression (SAGE) method, a total of 142 603 SAGE tags were sequenced and identified, representing 43 581 unique mRNA species. Comparison of expression patterns revealed that 53 cellular genes were differentially expressed upon HIV-1 infection. Northern blot and RT-PCR analyses confirmed the altered expression of the genes in both MOLT-4 and MT-4 cells. Up-regulated genes were mainly composed of transcription factors and genes related to T cell activation, whereas down-regulated genes were comprised of mitochondrial proteins, actin-related factors and translational factors. These findings indicate that persistent T cell activation, which may accelerate HIV-1 replication, and the disruption of cellular housekeeping genes including those involved in anti-apoptotic systems, may play an important role in HIV-1-induced pathogenesis.

Key words: HIV-1; Serial analysis of gene expression; Transcript; Virus-cell interaction

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) infection is composed of a host-parasite relationship in which virus utilizes the host cell's macromolecular machinery and energy supplies to produce progeny virus [1]. Inevitably, HIV-1 infection alters cellular physiological states leading to disturbance of immune responses, cell growth arrest, and cell death, where certain gene activities are considered to be crucial [2]. Studies on HIV-1 pathogenesis have recently been expanded to define the changes in gene expression occurring in infected cells in association with HIV-1 replication and apoptosis [3–5]. However, most of these studies have focused on only a limited number of biological parameters, even though the phenomena were clearly a part of a more complex network of related factors. To further understand the cellular events and the pathogenesis occurring after HIV-1 infection, it is essential to survey an overall differential gene expression pattern.

Herein, we used the serial analysis of gene expression (SAGE) method [6] to systematically analyze gene expression profiles in HIV-1-infected T cells. SAGE allows the simultaneous quantitative analysis of a large number of transcripts in cells or tissues without prior knowledge of genes [6–8]. For SAGE analysis, we used the human T cell line MOLT-4. MOLT-4 cells are well characterized and susceptible to HIV-1 infection [10], and can be maintained without any stimulation needed to facilitate HIV infection by agents such as phytohemagglutinin or anti-CD3 antibody, which may influence cellular gene expression [11]. We obtained a total of 142 603 SAGE tags and newly identified 53 cellular genes up- or down-regulated upon HIV-1 infection.

2. Materials and methods

2.1. Cell culture and HIV-1 infection

MOLT-4 and MT-4 cells were maintained at 37°C in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS). A total of 1×10^6 viable cells were mixed with 10^5 50% tissue culture infectious dose ($TCID_{50}$) of HIV-1_{NL4-3} and incubated for 2 h at 37°C [9]. Cells were washed twice with and resuspended in complete medium to 2.5×10^5 cells/ml. MOLT-4 cells were harvested at 96 h after infection in which HIV-1 antigen-positive cells were 53% of the total cells, the viability of cells was 92–95% and specific cytopathic effects were observed [10].

2.2. SAGE analysis

SAGE libraries were generated using 30 μg of total RNA from HIV-1_{NL4-3}- and mock-infected MOLT-4 cells as described previously [6], except that restriction enzymes *Rsa*I and *Bsm*FI were used as anchoring and tagging enzyme, respectively [12]. The concatemerized sequence tags were cloned into dephosphorylated pUC118 vectors. Recombinant plasmid DNAs were sequenced with Big Dye terminator kits (PE Applied Biosystems) using an ABI 377XL automated sequencer (PE Applied Biosystems). PROGENEX software (Fujiyakuhin Co., Saitama, Japan) was applied to extract and analyze SAGE tags [12]. The representative tag sequences were compared for homology against databases as described previously [13].

2.3. Northern blot analysis

Eight micrograms of total RNA was separated on a 1.5% agarose gel containing 0.66 M formaldehyde and transferred to a Duralon-UV membrane (Stratagene, La Jolla, CA, USA). The filters were baked at 80°C for 2 h. The cDNAs of ~300 bp were amplified with RT-PCR and labeled with [^{32}P]dCTP using a DNA labeling kit (Nippon Gene). Membrane filters were hybridized with labeled probes (1×10^6 cpm/ml) in the buffer (40% deionized formamide, 4×SSC, 10% Dextran sulfate, 1×Denhardt's solution, 40 μg/ml sonicated and denatured salmon sperm DNA, 0.1% SDS, 20 mM Tris (pH 7.5)) at 42°C for 16 h. The filters were washed twice for 15 min at room temperature and once for 30 min at 56°C with 2×SSC containing 0.1% SDS, and exposed using a Fuji Imaging Plate [13]. Densitometric analysis was performed using a BAS2000 system (Fuji Photo Co., Tokyo, Japan).

2.4. Kinetics of infection.

Mock- and HIV-1_{NL4-3}-infected MT-4 cells were harvested at several time points for 96 h post-infection (p.i.) (see Fig. 2). Viable cells were counted by the trypan blue dye exclusion method. Virus production in culture supernatant from HIV-1-infected cells was monitored by an enzyme-linked immunosorbent assay (ELISA) specific to the

*Corresponding author. Fax: (81) (3) 5803-0124.
E-mail: yamamoto.mmb@med.tmd.ac.jp

Abbreviations: HIV, human immunodeficiency virus; SAGE, serial analysis of gene expression

PII: S0014-5793(99)01526-4

HIV-1 p24-gag antigen (Coulter, Hialeah, FL, USA) according to the manufacturer's instruction.

3. Results

3.1. SAGE analysis

A summary of transcript abundance and corresponding gene representation is provided in Table 1. Among 142 609 transcripts representing 43 581 unique genes, approximately half were derived from the population of HIV-1- and mock-infected MOLT-4 cells.

Comparison of the two SAGE profiles indicated that the great majority of genes were expressed at similar levels irrespective of virus infection. However, approximately 200 genes were expressed at significantly different levels (by five-fold or more) in HIV-1-infected MOLT-4 cells. Of these differentially expressed genes, we selected 57 sequence tags that matched known genes and EST entries. Among the up-regulated genes, the four that only appeared in the HIV-1-infected cell profile represented HIV-1 transcripts (Table 2). The remaining 22 genes up-regulated upon HIV-1 infection were arbitrarily subdivided into four groups (Table 2): (1) genes related to cell activation and signaling pathways, (2) transcription factors, (3) interferon-induced genes and (4) miscellaneous genes. Likewise, 31 down-regulated genes were classified into four groups (Table 3): (1) mitochondrial proteins and antioxidants, (2) actin-related factors, (3) translational factors and (4) miscellaneous genes. Overall, the up-regulated genes were mainly comprised of genes that accelerated HIV-1 replication, whereas down-regulated genes were involved in anti-apoptotic cell defense and regulation of basic cellular functions.

To test the reliability of SAGE results, we examined the expression of 10 arbitrarily selected genes by Northern blot

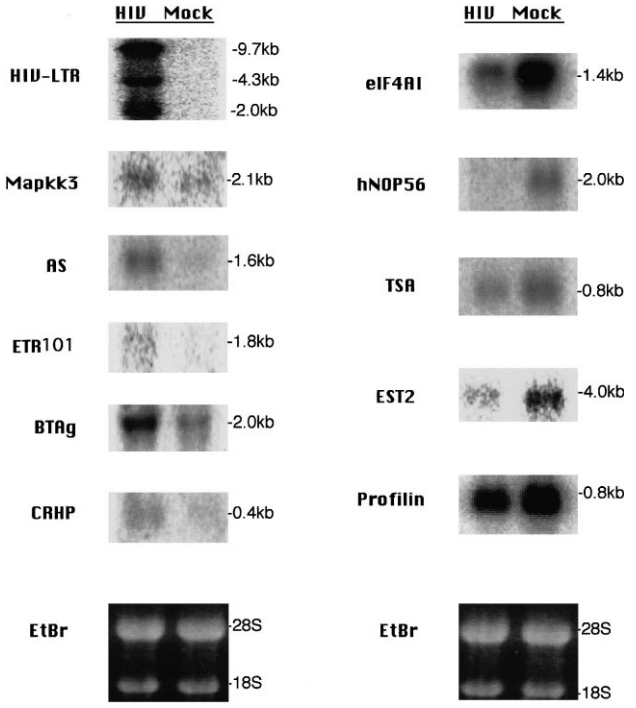

Fig. 1. Northern blot analysis of genes differentially expressed in HIV-1_{NL4-3}-infected MOLT-4 cells. HIV-LTR, HIV-1 long terminal repeat; MAPKK3, mitogen-activated protein kinase kinase 3; AS, argininosuccinate synthetase; ETR101, transcriptional factor ETR101; BTAg, breast tumor autoantigen; CRHP, cysteine-rich heart protein; eIF4AI, eukaryotic initiation factor 4AI; NOP56, nucleolar protein NOP56; TSA, thiol-specific antioxidant; EtBr, ethidium bromide.

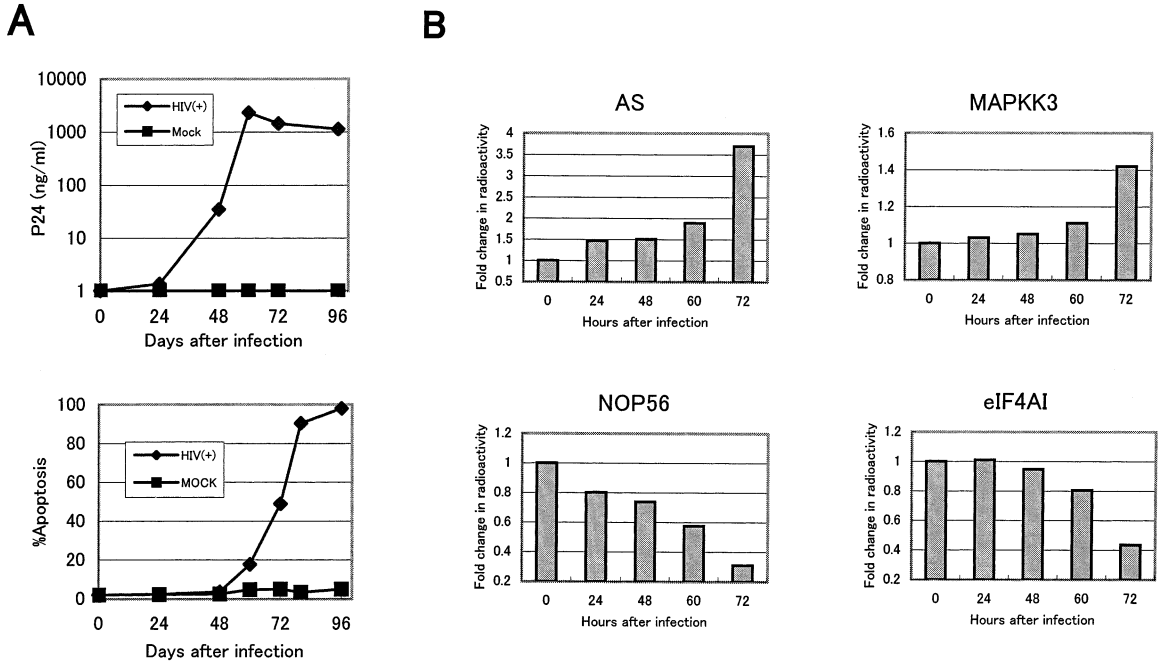

Fig. 2. A: Kinetics of virus replication ($p24^{gag}$ antigen in culture fluid) (upper) and apoptosis levels (lower) in HIV-1_{NL4-3}-infected MT-4 cells. $P24^{gag}$ antigen in culture fluid was measured by an ELISA specific for the HIV-1 $p24^{gag}$ antigen (Coulter, Hialeah, FL, USA). Degree of apoptosis was analyzed using propidium iodide staining and flow cytometry. B: Kinetics of mRNA levels of selected genes in HIV-1_{NL4-3}-infected MT-4 cells. Cells were collected at 24, 48, 60, 72 h after infection. Signals were quantitated densitometrically normalized to ribosomal protein L32, and expressed relative to the signals in the uninfected cells. Gene descriptions were the same as in Fig. 1.

Table 1
Summary of SAGE analysis

	HIV	Mock	Overall
Total number of clones	6 245	6 461	12 706
Total number of tags	71 462	71 147	142 609
Genes[a]	24 872	24 741	43 581

[a]Indicates the number of different genes represented by total SAGE tags analyzed.

analysis. The results of Northern analysis substantiate the differential expression of the genes in infected MOLT-4 cells (Fig. 1). RT-PCR also confirmed significant differences in the expression of whole genes in Tables 2 and 3 between the samples (data not shown).

3.2. Kinetics of mRNA expression associated with virus replication and apoptosis

To address whether the genes detected in the MOLT-4 cell line are also dysregulated in other $CD4^+$ T cell lines, and to investigate the kinetics of these changes, we arbitrarily selected four genes and performed Northern blot analysis using MT-4 cells. Kinetic study showed that almost all MT-4 cells were infected with HIV-1 within a few days followed by severe apoptotic cell death and extremely high levels of virus replication (Fig. 2A). Northern blot analysis revealed that the expression levels of nucleolar protein NOP56 and eukaryotic initiation factor (eIF)-4AI gradually decreased during the process of infection, while those of argininosuccinate synthetase (AS) and mitogen-activated protein kinase kinase 3 (MAPKK3) increased rapidly at 72 h post-infection (Fig. 2B). These results indicate that the altered expression of the genes identified by SAGE was closely correlated with the time course of viral replication and apoptosis. This confirms the validity and feasibility of the present study.

4. Discussion

4.1. Genes related to the HIV-1 replication signals are overexpressed

Consistent with the notion that HIV-1 deregulates genes involved in HIV replication [14], a number of genes encoding accelerators for viral and cell growth are stimulated in HIV-1-infected T cells. We grouped these genes into five groups.

The first group includes genes linked to cell activation and related genes involved in signaling pathways. Peripheral blood micronuclear cells from asymptomatic patients showed a significantly increased level of tumor necrosis factor α (TNF-α)-receptor-positive cells compared with controls, although this was not the case in AIDS patients [15]. Several reports also

Table 2
Up-regulated genes in HIV-1-infected T cells

SAGE tag	Gene description	Accession number	H/M[a]
0. HIV transcripts			
TGGGTCTCTCTGGT	HIV-1 transcript	Z11530	92/0
CTGAGGTGTGACTG	HIV-1 transcript	Z11530	9/0
CGTCAGCGTCATTG	HIV-1 transcript	Z11530	9/0
CCACAGACCCCAAC	HIV-1 transcript	Z11530	7/0
1. Cell activation and signaling pathway			
CAGCAGGCAGAGCC	Mitogen-activated protein kinase kinase 3 (MAPKK3)	D87116	37/7
TCAGGAGGCTGAGG	Tumor necrosis factor receptor 75 kDa	S63368	35/7
AGGCGCTAATTGTT	Argininosuccinate synthetase (AS)	X01630	15/0
TGTGGGCTGTGCTG	Transcription factor ETR101	M62831	13/1
CAGAGGATGGTGAG	Fibroblast growth factor receptor (FGFR)	M60485	12/1
GCACCCGCTGGGCA	Lymphocyte activation antigen 4F2 large subunit	J03569	9/1
TAGCTGTGTGTTCT	Ca channel B3 subunit (CAL Bet 3)	L27584	6/0
AGACGGTGTGGGGG	Leukosialin (CD43)	M61827	5/0
2. Transcription factors			
TGAGACAGGGTGCT	Helix-loop-helix zipper protein	M77476	21/4
CAGGGCCATGCAGG	Basic leucine zipper transcription factor MafG	U84249	11/1
TGAGATGTGGCTGG	Zinc finger protein (ZNF139)	U09848	6/0
CCCTCTGACCCACC	Ets domain protein ERF	U15655	5/0
AGCTCCGGACTCTT	GATA-3 enhancer binding protein	M69106	5/0
3. IFN-induced genes			
GGCCTCAAGCCCCT	Interferon-induced 17/15 kDa protein	M13755	33/6
CAGGGCAAGAAGCC	Interferon-inducible protein (IP-30)	J03909	12/1
AATGCTGCTGCCTT	Putative interferon-related protein (SM15)	U09585	6/0
4. Miscellaneous genes			
CAGTGTGTGTTGAT	EST 1	W86328	20/2
TGTCCATCTGCCTG	Rapamycin and FK506 binding protein	M75099	10/0
AGCCCCAGATGGGA	HIV-1 promoter region chimeric mRNA	U19178	5/0
CCTGTGTTTTACCT	Breast tumor autoantigen	U24576	5/0
TTCGCCGAGAGGGT	Cysteine-rich heart protein (CRHP)	U09770	5/0
CCGCCCATGAACCC	Moesin-ezrin-radixin-like protein	L11353	5/0

[a]H/M values indicate the frequency with which each tag appeared in the profiles from HIV (H)- and mock (M)-infected MOLT-4 cells. The frequency of each tags was calculated within a total population of 71 462 tags and 71 147 tags sequenced from HIV-1- and mock-infected MOLT-4 cells, respectively.

Table 3
Down-regulated genes in HIV-1-infected T cells

SAGE tag	Gene description	Accession number	H/M[a]
1. Mitochondrial proteins and anti-oxidants			
TATACTTCACAACA	Mitochondrion cytochrome *b*	U09500	7/50
CCAGTGATCCCCAC	Cytochrome *c* oxidase subunit Vib (COXVib)	X54473	1/30
TGTCTCTCTCCTTG	ATP synthase β subunit	M27132	2/19
CACTGCTAATAAAT	Cytochrome *c* oxidase subunit IV (COX IV)	M21575	2/19
ACATAAGTTATTTC	ADP/ATP carrier protein	J02683	1/17
CAGGAAAGAGGATA	Mitochondrial aspartate aminotransferase	M22632	0/16
CTGGATGAAGCATA	Glutathione *S*-transferase homolog	U90313	2/16
ACAGACGAGCATGG	Thiol-specific antioxidant	X82321	0/12
TGAGACCTAGAGTC	ADP/ATP translocase	J03592	0/11
TCCTATGCAATATT	ADP-ribosylation factor 1	M84326	1/11
AAACCCACGTTTTG	Mitochondrial 75 kDa iron sulfur protein	X61100	0/9
TTTGCTCCATTGTT	150 kDa oxygen-regulated protein (ORP150)	U65785	0/8
2. Actin-related factors			
TGACCTCGTCTGTC	Profilin	J03191	15/66
TCTGGTGAGTCACC	GTP binding protein (rhoC)	L25080	2/32
GGGAGTTTCTTGGT	Arp2/3 protein complex subunit p34-Arc (ARC34)	AF006085	1/7
CATACATGAGTTAT	Actin-related protein Arp2 (ARP2)	AF006082	0/6
ACAATCATTTAATA	Rho GDP-dissociation inhibitor 2	X69549	0/5
3. Translational factors			
CCCTGGCCGTGTGT	Eukaryotic initiation factor 4AI	D13748	7/50
TCCAGAGGAGTGTG	Nucleolar protein hNop56	Y12065	2/17
CCAAGTCTTACGTT	Inducible poly(A) binding protein	U33818	0/10
TGCTTCCAAGCAGC	DEAD box protein family	X70649	1/8
TCCTGTTTGGAAGT	Translation initiation factor nuk34	X79538	0/7
TACGTGAAACTGAA	Nuclear RNA helicase	Z37166	1/6
ACTTGCTGGTCTAG	Translation initiation factor 3	U94855	0/5
4. Miscellaneous genes			
CGATCCTGAGACCT	Ornithine decarboxylase (ODC1)	M16650	2/24
GCAAAGAGAACCAG	Cyclin A/CDK2-associated p19 (Skp1)	U33760	1/17
TAACTTTCCTTCAT	Interleukin 2 receptor (IL2RG) γ chain	D11086	2/12
TATAAGTAGTTGGT	Prothymosin α	M14630	1/12
GTGCTAACAGGCTC	Transferrin receptor	X01060	1/11
CCTGGGGAATCAAC	EST 2	AA825204	1/10
TGTCTGGCTTGGAT	RanGTP binding protein 5	Y08890	1/8
TTGGTAAGAGGGAG	Down syndrome critical region protein (DSCR1)	U28833	0/6
TTCGAATTTGAGTT	TGF-β receptor interacting protein 1	U36764	1/5

[a]Conditions are as described in Table 2.

showed that persistent TNF-α activation was correlated with disease progression in patients infected with HIV-1 [15,16]. Furthermore, TNFs apparently activate HIV-1 replication in vitro [16]. These data suggest that persistent activation of the TNF system may play important roles in HIV-1 replication and virus-induced pathogenesis. MAPKK3 was up-regulated upon HIV-1 infection. HIV-1 envelope glycoproteins rapidly activate the MAPK pathway and the binding of nuclear transcription factors (AP-1, NF-κB and C/EBP) and stimulate expression of cytokine and chemokine genes [17]. These events may lead to the aberrant expression of inflammatory genes and may contribute to HIV-1 replication as well as to dysregulation of the immune system.

The second group of genes are related to transcriptional factors and stimulators of HIV-1 transcription. Proteins containing a basic region helix-loop-helix zipper motif have DNA binding activity and have been predicted to mediate protein-protein interactions with myc and fos [18]. Since expression of c-myc is essential for HIV-1 infection in T cells [19], it is possible that the activation of helix-loop-helix zipper protein by HIV-1 infection may play a role in viral replication and apoptosis. Expression of the transcription factor GATA-3 was also up-regulated upon infection. The GATA-3 gene product binds specifically to the enhancer elements of TCR genes [20]. GATA3 also binds to six GATA-3 binding sites in the U3 region of the HIV-1 LTR and enhances LTR-mediated transcription activity [21]. The up-regulation of GATA-3 in HIV-1-infected T cells may lead to induction and augmentation of HIV-1 replication in addition to its normal role in T cell gene expression.

4.2. Genes encoding anti-apoptotic proteins are down-regulated

Our SAGE analysis also revealed a number of genes that were markedly inhibited by HIV-1, which we subdivided into four groups.

The first group included mitochondrial proteins and anti-oxidants [22]. Indeed, HIV-1 infection is characterized by significant impairments of antioxidative defenses [23]. Oxidative stress has also been proposed to be involved in immunologic defects as well as in lymphocyte cell death and viral replication in HIV-1-infected patients [24]. Thiol-containing antioxidants have been shown to have beneficial effects on $CD4^+$ T lymphocyte survival and to inhibit apoptosis and HIV-1 replication most probably through blocking the activation of NF-κB [23]. It is thus possible that a decrease in the levels of thiol-specific antioxidant upon HIV-1 infection results in the generation of reactive oxygen species (ROS) and apoptosis [25]. Down-regulation of cytochrome *c* oxidase (COX) is also reported in *Mycobacterium tuberculosis* infection in macrophages [26]. Down-regulation of COXs upon infection with HIV-1 may result in mitochondrial respiratory insufficiency

and general interference with oxidative metabolism leading to apoptotic cell death [22].

Secondly, some genes relevant to actin polymerization and related molecules were down-regulated. Polymerization of actin has been implicated in reverse transcription [27] and directional budding [28] of HIV-1 from infected cells. Down-regulation of actin-related factors may contribute to a host cell defense mechanism which blocks virus replication and budding as well as cellular malfunction accompanied by the disorganization of cytoskeleton, cell cycle and adhesion molecules in HIV-1-infected T cells [29].

The third group of genes are those related to cellular translation [30]. This indicates that HIV-1 infection directly causes the breakdown of cellular replication systems and it might therefore be involved in T cell depletion.

This is the first report that demonstrates the systematic pattern of gene expression in HIV-infected cells by SAGE analysis. To provide a simplified picture of changes in gene expression with HIV infection by SAGE, the present study used a monoclonal T cell line and a monoclonal HIV strain (HIV-1_{NL4-3}), and therefore the results are only preliminary. Further studies using other samples such as those from HIV-1-infected individuals at various stages of infection are necessary.

In summary, we have shown that at least 53 cellular genes are up- or down-regulated in HIV-1-infected T cell lines by SAGE analysis. Further study of the genes identified in this study should provide new and useful insights into virus-host cell interactions, the pathogenesis of and potential treatments for HIV-1 infection.

Acknowledgements: The authors thank Masaaki Arai, Kenji Tanaka, Masahiro Shuda and Mizue Shichita for technical assistance. We would like to acknowledge the Human Genome Center of the Institute of Medical Science, The University of Tokyo for providing valuable databases. A.R. is a fellow of the Japan Society for the Promotion of Science. This work was supported by grants from Health Sciences of Organization for Drug ADR Relief, R&D Promotion and Product Review of Japan and CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation (JST).

References

[1] Fauci, A.S. (1996) Nature 384, 529–534.
[2] Pantaleo, G. and Fauci, A.S. (1996) Annu. Rev. Microbiol. 50, 825–854.
[3] Kaplan, D. and Sieg, S. (1998) J. Virol. 72, 6279–6289.
[4] Hashimoto, F., Oyaizu, N., Kalyanaraman, V.S. and Pahwa, S. (1997) Blood 90, 745–753.
[5] Scheuring, U.J., Corbeil, J., Mosier, D.E. and Theofilopoulos, A.N. (1998) AIDS 12, 563–570.
[6] Velculescu, V.E., Zhang, L., Vogelstein, B. and Kinzler, K.W. (1995) Science 270, 484–487.
[7] Zhang, L., Zhou, W., Velculescu, V.E., Kern, S.E., Hruban, R.H., Hamilton, S.R., Vogelstein, B. and Kinzler, K.W. (1997) Science 276, 1268–1272.
[8] Hibi, K., Liu, Q., Beaudry, G.A., Madden, S.L., Westra, W.H., Wehage, S.L., Yang, S.C., Heitmiller, R.F., Bertelsen, A.H., Sidransky, D. and Jen, J. (1998) Cancer Res. 58, 5690–5694.
[9] Kawano, Y., Tanaka, Y., Misawa, N., Tanaka, R., Kira, J.I., Kimura, T., Fukushi, M., Sano, K., Goto, T., Nakai, M., Kobayashi, T., Yamamoto, N. and Koyanagi, Y. (1997) J. Virol. 71, 8456–8466.
[10] Kikukawa, R., Koyanagi, Y., Harada, S., Kobayashi, N., Hatanaka, M. and Yamamoto, N. (1986) J. Virol. 57, 1159–1162.
[11] Kern, J.A., Reed, J.C., Daniele, R.P. and Nowell, P.C. (1986) J. Immunol. 137, 764–769.
[12] Ryo, A., Kondoh, N., Wakatsuki, T., Hada, A., Yamamoto, N. and Yamamoto, M. (1999) Anal. Biochem. (in press).
[13] Ryo, A., Kondoh, N., Wakatsuki, T., Hada, A., Yamamoto, N. and Yamamoto, M. (1998) Nucleic Acids Res. 26, 2586–2592.
[14] Perkins, N.D., Agranoff, A.B., Duckett, C.S. and Nabel, G.J. (1994) J. Virol. 68, 6820–6823.
[15] Aukrust, P., Liabakk, N.B., Muller, F., Lien, E., Espevik, T. and Froland, S.S. (1994) J. Infect. Dis. 169, 420–424.
[16] Matsuyama, T., Hamamoto, Y., Soma, G., Mizuno, D., Yamamoto, N. and Kobayashi, N. (1989) J. Virol. 63, 2504–2509.
[17] Popik, W., Hesselgesser, J.E. and Pitha, P.M. (1998) J. Virol. 72, 6406–6413.
[18] Blanar, M.A. and Rutter, W.J. (1992) Science 256, 1014–1018.
[19] Sun, Y. and Clark, E.A. (1999) J. Exp. Med. 189, 1391–1398.
[20] Marine, J. and Winoto, A. (1991) Proc. Natl. Acad. Sci. USA 88, 7284–7288.
[21] Yang, Z. and Engel, J.D. (1993) Nucleic Acids Res. 21, 2831–2836.
[22] Kroemer, G., Zamzami, N. and Susin, S.A. (1997) Immunol. Today 18, 44–51.
[23] Sandstrom, P.A., Murray, J., Folks, T.M. and Diamond, A.M. (1998) Free Radical Biol. Med. 24, 1485–1491.
[24] Schreck, R., Rieber, P. and Baeuerle, P.A. (1991) EMBO J. 10, 2247–2258.
[25] Kowaltowski, A.J., Netto, L.E. and Vercesi, A.E. (1998) J. Biol. Chem. 273, 12766–12769.
[26] Ragno, S., Estrada-Garcia, I., Butler, R. and Colston, M.J. (1998) Infect. Immun. 66, 3952–3958.
[27] Bukrinskaya, A., Brichacek, B., Mann, A. and Stevenson, M. (1998) J. Exp. Med. 188, 2113–2125.
[28] Iyengar, S., Hildreth, J.E. and Schwartz, D.H. (1998) J. Virol. 72, 5251–5255.
[29] Howard, T.H. and Watts, R.G. (1994) Curr. Opin. Hematol. 1, 61–68.
[30] Gautier, T., Berges, T., Tollervey, D. and Hurt, E. (1997) Mol. Cell. Biol. 17, 7088–7098.