Title

A screening for DNA damage response molecules that affect HIV-1 infection

Authors

Noriyoshi Yoshinaga[1], Keisuke Shindo[1], Yusuke Matsui[1], Yoko Takiuchi[1], Hirofumi Fukuda[1], Kayoko Nagata[1], Kotaro Shirakawa[1], Masayuki Kobayashi[1], Shunichi Takeda[2] and Akifumi Takaori-Kondo[1]

Affiliations

1) Department of Hematology and Oncology, and 2) Department of Radiation Genetics, Graduate School of Medicine, Kyoto University

Correspondence

Keisuke Shindo, Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaracho, Sakyo-ku, Kyoto, 606-8507, Japan.

Phone: +81-75-751-4964; FAX: +81-75-751-4963

E-mail: shind009@kuhp.kyoto-u.ac.jp

Abstract

Host DNA damage response molecules affect retroviral infection, as DNA intermediates of the viruses play essential roles in the viral life cycles. Although several such molecules have been reported, interactions between HIV-1 and host DNA damage response molecules have not been fully elucidated. To screen DNA damage response molecules that might affect HIV-1 infection, a set of 32 DNA-repair-deficient DT40 isogenic mutant cells were tested for HIV-1 infectivity. Seven out of the 32 clones showed less than 50% infectivity compared to parental DT40 cells, implying that DNA repair molecules deficient in these cells might support HIV-1 infection. Of these, *EXO1 -/-*, *TP53BP1 -/-* and *WRN -/-* cells showed more than twofold accumulation of two long terminal repeat circles and less than 50% integrated proviral DNA in quantitative-PCR analyses, indicating that the integration step is impaired. *RAD18 -/-* cells showed twofold higher HIV-1 infectivity and increased reverse transcription products at earlier time points, suggesting that RAD18 suppresses reverse transcription. The HIV-1 suppressive effects of RAD18 were confirmed by over-expression and knockdown experiments in human cells. L274P, a DNA-binding-impaired mutant of RAD18, showed impaired HIV-1 suppression and DNA binding, suggesting that binding HIV-1 DNA intermediates is critical for RAD18 to suppress reverse transcription and HIV-1 infection. Our data help understand interactions between host DNA damage response molecules and viral DNA.

Keywords: retrovirus; host factors; reverse transcription; RAD18; virus-host interactions; DNA damage

response

Introduction

DNA repair system is fundamental to cells to maintain genetic materials, which encode molecules required for their physiological activities. Lack or dysfunction of a single DNA repair molecule can cause accumulation of DNA damage and consequent cell death or tumorigenesis. Various DNA lesions can be detected and modified by DNA repair system. For example, DNA double-strand breaks (DSBs) are recognized and ligated by homologous recombination or non-homologous end joining pathways [1]. Mismatch repair contributes to genome stability by excising DNA mismatches introduced by DNA polymerase [2]. Translesion synthesis is a DNA damage tolerance (DDT) process that allows the DNA replication machinery to replicate past DNA lesions [3].

Human immunodeficiency virus type 1 (HIV-1), which possesses RNA genome, establishes infection by integrating double-stranded viral DNA into host genome after entry to target cells, and integrated proviral DNA is transcribed and then translated for the viral replication using various host factors [4,5,6,7,8]. In the process of HIV-1 infection, various forms of viral DNA are produced in newly infected target cells, and several of those might be recognized by host DNA repair molecules, which can positively or negatively affect life cycles of HIV-1 [9,10,11]. For example, a couple of reports implicated the flap endonuclease 1 (FEN1) plays roles in late steps of HIV-1 integration [12,13], and RAD52, a homologous

recombination factor, was reported to suppress HIV-1 infection by competing with active integration complex [14]. Although several of such molecules have been identified, interactions between HIV-1 DNA intermediates and host DNA repair molecules, and implications of those interactions remain unclear.

Using the DNA-repair-deficient DT40 isogenic mutant cells, we have found several candidate molecules that influence retroviral infection. RAD18 suppressed HIV-1 infection and POLZ, EXO1, TP53BP1, FEN1, ATM and WRN supported HIV-1 replication. RAD18 is a ubiquitin ligase that is involved in DDT pathway promoting PCNA mono-ubiquitination and also stimulates homologous recombination to repair DSBs [15]. A previous report showed that over-expression of RAD18 impaired HIV-1 infection [16], however, underlying mechanisms remain mostly unknown. Here we show that RAD18 suppresses HIV-1 in the step of reverse transcription, and DNA binding is involved in the suppression. Overexpression and knockdown experiments confirmed the suppressive effects of RAD18 on HIV-1 infection, and a DNA-binding-impaired mutant of RAD18 showed decreased HIV-1 suppression. Immunoprecipitation of RAD18 followed by quantitative PCR analyses confirmed RAD18 binding to HIV-1 DNA intermediates. Our data provide additional insights into how host DNA damage response pathways affects the life cycle of HIV-1.

Materials and Methods

Plasmid construction

Human RAD18 expression plasmid was generated by amplifying coding sequence from cDNA of 293T

cells using primers 5'-CAGCATCCTCGGGAGCGA-3' and 5'-AAAGTCAGCAAAAGCCCACA-3', and inserting it into pcDNA3 (Invitrogen) at Xba I/Kpn I sites. The expression vectors for RAD18 mutants were generated by a PCR-based method using properly modified primers. pNL4-3/*ΔEnv-Luc* vector was previously described [17]. pDON/*Luc*, a murine retroviral vector with luciferase reporter, pMLV *gag/pol*, and pVSV-G were previously described [18].

Cell culture

293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS) and penicillin, streptomycin and glutamine (PSG). DT40 cells were maintained in RPMI1640 medium supplemented with 10% FBS, 1% chicken serum, 10^{-5} M β-mercaptoethanol and PSG. Jurkat cells were maintained in RPMI1640 medium supplemented with 10% FBS and PSG.

Preparation of viruses and single-cycle infection

VSV-G pseudotyped HIV-1 particles with a luciferase reporter gene were produced by cotransfection of 4 μg pNL4-3/*ΔEnv-Luc* and 1 μg pVSV-G into 293T cells on a 10 cm-dish at 50% confluency using X-tremegene HP DNA transfection reagent (Roche) according to manufacturer's instruction. MLV particles were similarly produced by cotransfection of 2.5 μg pDON/*Luc*, 2 μg pMLV *gag/pol* and 0.5 μg pVSV-G. After 48 hours, virus-containing supernatants were harvested through PVDF filter with 0.45μm pores

(Millipore), and challenged to DT40 cells, 293T cells, or Jurkat cells. After 24 hours, cells were lysed with Passive lysis buffer (Promega) and luciferase activity was determined by luminometer (2030 Arvo X, Perkin Elmer) using Luciferase Assay System (Promega).

Quantitative PCR.

To measure time course of HIV-1 late reverse transcripts, each mutant and parental DT40 cells were plated in 12-well plates at a density of 20,000 cells/well, and the following day, infected with DNase-treated VSV-G pseudotyped HIV-1 for 2 hours, then cells were washed with PBS and fed with complete medium. Cells were harvested at 4, 8, 12, and 24 hours post-infection, and total DNA was extracted by using Quick Gene DNA whole blood kit (Fuji Film). Quantitative PCR for late RT products was performed as previously described [19]. Briefly, real-time PCR was performed with Sybr premix Ex Taq II (Takara) according to manufacturer's protocol with 50 ng of total DNA and primers 5'-TGTGTGCCCGTCTGTTGTGT-3' and 5'-GAGTCCTGCGTCGAGAGATC-3'. *Histone H4* was simultaneously measured using primers 5'-TGCGCGACAACATCCAGGGCATCAC-3' and 5'-GTGACCGTCTTCCTCTTGGCGTGCTC-3' for normalization. To measure 2-LTR circle DNA, 50 ng of total DNA from cells 24 hours post-infection was used with primers 5'-TGGTTAGACCAGATCTGAGCCT-3' and 5'-GTGAATTAGCCCTTCCAGTACTGC-3', as preciously described [19]. To quantify integrated proviral DNA of HIV-1 from chicken DT40 cells, PCR between HIV-1 and chicken repeat 1 sequences was used

[20,21]. 50 ng of total DNA from cells 24 hours post-infection, and primers 5'-ATAGAATCATAGAATGGCTG-3' (chicken repeat 1) and 5'-CTATCCTTTGATGCACACAATAGAG-3' (HIV-1 gag) were used for the first PCR, and 5'-TGTGTGCCCGTCTGTTGTGT-3' (HIV-1 LTR) and 5'-GAGTCCTGCGTCGAGAGATC-3 (HIV-1 LTR) for the second PCR. PCR standards were obtained from serially diluted genomic DNA isolated from 293T cells, and the data were normalized to the corresponding values of *histone H4*.

RNA interference

Human RAD18 knockdown cells were generated by transduction of small interfering RNA (siRNA) or short hairpin RNA (shRNA). A total of 5×10^6 Jurkat cells were electroporated with 20 pmol/μl of either nontargeted siRNA (MISSION® siRNA Universal Negative Control, Sigma-Aldrich) or RAD18-specific siRNA (siGenome Smartpool, Thermo Scientific) using the Amaxa human T-cell nucleofector kit (Lonza, Basel, Switzerland) as per manufacturer protocols. We modified the psicoR-mCherry vector for delivery of shRNA against *RAD18* to 293T cells. The shRNA sequence used was 5'-GAGCATGGATTATCTATTCAA-3', which has been previously described [22]. The RAD18 protein levels in the transduced Jurkat T cells and 293T cells were verified by immunoblotting.

Immunoblotting

A total of 5×10^6 cells was lysed with ice-cold lysis buffer (MPER, Thermo Scientific) containing 1 mM phenylmethylsulfonyl fluoride, phosphatase inhibitor cocktail (Roche), and protease inhibitor cocktail (Nacalai Tesque), followed by centrifugation at 13,000 rpm for 15 min at 4 °C. The supernatants were mixed with an equal volume of 2x sample buffer (Bio-Rad) containing β-mercaptoethanol (Nacalai) and were treated for 5 min at 100 °C. We used a standard chemiluminescence protocol for immunoblotting with PVDF membrane (Millipore). Antibodies used for immunoblotting were rabbit anti-RAD18 (Cell Signaling), rabbit anti-myc serum (C3956, Sigma) and mouse anti–β-actin monoclonal antibody (AC-15, A5441, Sigma).

Immunoprecipitation

For co-immunoprecipitation to test interaction of HIV-DNA and RAD18, 293T cells were transfected with the expression vector for RAD18 wild-type or L274P mutant, or an empty vector as control, and infected with HIV-1. After 24 hours, cells were lysed with co-IP buffer (25 mM HEPES, pH 7.4, 150 mM NaCl, 0.1% Triton X-100, 1mM EDTA, 1mM MgCl2) supplemented with protease inhibitor cocktail (Nacalai), centrifuged at 20,000 × g for 10 minutes, and supernatant was mixed with 2 μg anti-myc rabbit serum (Sigma) for 1 hour, and then mixed with 20 μl protein A sepharose (Pharmacia) for 1 hour. Beads were washed with co-IP buffer 3 times and collected for PCR to test HIV-DNA binding. The primers used were LTR forward, 5’-TGTGTGCCCGTCTGTTGTGT-3’; HIV-1 LTR reverse, 5’-

GAGTCCTGCGTCGAGAGATC-3'. The other bound protein was eluted with 1x SDS sample buffer. Samples were analyzed by immunoblotting as described above.

Results

Identification of the host factors that affect HIV and MLV infectivity in DT40 cells

To identify the DNA damage response molecules that affect HIV-1 replication, a set of 32 DNA-repair-deficient DT40 cell clones that consist of 28 clones with deletion of individual DNA repair genes, one clone with one amino acid substitution of a single gene, and three clones with double gene deletion (Supplementary Table 1) were tested for infectivity of VSV-G pseudotyped NL4-3 with a luciferase reporter. The infectivity of HIV-1 varied among cell lines; *POLZ -/-*, *EXO1 -/-*, *TP53BP1 -/-*, *FEN1 -/-*, *POLH/Z -/-*, *ATM -/-* and *WRN -/-* cells showed less than 50% infectivity of that in parental DT40 cells, and *RAD18 -/-* cells showed more than twofold higher infectivity (Figure 1, dark blue bars). These results indicate that several DNA repair molecules that are deficient in these cells actually affect the process of HIV-1 life cycle. To help postulate how these molecules affect HIV-1 infection, we next performed similar experiments with MLV-based reporter virus. MLV infectivity was reduced in *POLZ -/-*, *TP53BP1 -/-*, *FEN1 -/-*, *ATM -/-* and *RAD18 -/-* cells as seen in the infectivity of HIV-1 (Figure 1, light blue bars), suggesting that the DNA

repair molecules deficient in these cells might affect broad types of retroviruses at common steps of retroviral replication. On the other hand, MLV infectivity in *WRN -/-* cells showed comparable infectivity to that in parental DT40 cells, suggesting that WRN affects specifically HIV-1 at the step present only in HIV-1 replication.

RAD18 inhibits reverse transcription of retroviruses

We selected eight mutant clones displaying either less than 50% or more than 200% infectivity of HIV-1 in comparison with the parental cells for further investigation. To elucidate in what steps in the viral life cycle these candidate molecules were involved, cells were infected with HIV-1, and late reverse transcription (RT) product of HIV-1 was measured at several time points by quantitative-PCR. *RAD18 -/-* cells contained about 5 and 1.6 times higher late RT products than parental cells at 4 and 8 hours after infection, respectively (Figure 2). These results suggest that higher infectivity of HIV-1 in *RAD18 -/-* cells is due to higher efficiency of RT in these cells compared with the parental cells and that RAD18 is likely to inhibit reverse transcription of HIV-1. On the other hand, *ATM -/-*, *EXO1 -/-*, *FEN1 -/-*, and *POLZ -/-* cells contained lower late RT products, suggesting that DNA repair molecules deficient in these cells might be supportive for reverse transcription of HIV-1. *TP53BP1 -/-* and *WRN -/-* cells showed slightly less RT product (Figure 2), but that seems not to be mainly responsible for lower infectivity in these cells, comparing infectivity and

RT results.

EXO1, TP53BP1 and WRN promote integration of viral reverse transcription products

To further analyze these candidate molecules, we also quantified the numbers of integrated provirus and two long terminal repeat (2-LTR) circle DNA of HIV-1 in the selected clones (Figure 3). The copy number of 2-LTR circle production was increased in *EXO1 -/-*, *TP53BP1 -/-* and *WRN -/-* cells, whereas that of integrated proviral DNA was decreased in these cells, indicating that EXO1, TP53BP1, and WRN may help integration of proviral DNA into host genome. *FEN1 -/-* cells showed similar results with these cells, consistent with the report that FEN1 can catalyze the late steps of retroviral DNA integration [13]. *RAD18 -/-* cells contained more than twice higher copy number of integrated HIV-1 proviral DNA in comparison with the parental cells, which data is consistent with the results that RT product was increased in these cells (Figures 2 and 3). Integrated proviral DNA was slightly decreased in *ATM -/-* and *POLZ -/-* cells, likely reflecting decreased RT products (Figures 2 and 3).

DNA binding is required for RAD18 to suppress HIV-1 infection

One report showed that over-expression of RAD18 makes human HeLa cells more resistant to HIV-1

infection [16], but the mechanism by which RAD18 suppresses HIV-1 infection remains unclear. To confirm that RAD18 suppresses HIV-1 infection in human cells, siRNA against human RAD18 was introduced into 293T and Jurkat cells, and HIV-1 infectivity of these cells were then tested. RAD18 protein level was reduced more than 90% by siRNA, and HIV-1 infectivity was increased about 1.7-fold and 1.5-fold in 293T cells and Jurkat cells, respectively (Figure 4a). These results suggest that endogenous RAD18 negatively affects HIV-1 infection in human cells. RAD18 is a multi-functional-domain protein bearing several structural motifs; RING domain has E3 ligase activity, zinc finger domain has an affinity for different poly-ubiquitin chains, and SAP domain is required for binding to DNA [23]. To determine which domain of RAD18 is required for suppressing HIV-1 infection, we generated expression plasmids for RAD18 mutants carrying a mutation in the RING domain (C28F), the zinc finger domain (D221A), or SAP domain (L274P) (Figure 4b). 293T cells were transiently transfected with various amounts of the expression plasmid for RAD18 wild-type or one of the mutants, and then infected with the luciferase-reporter HIV-1. Over-expression of RAD18 wild-type, C28F and D221A comparably reduced HIV-1 infectivity in dose-dependent manners, but that of L274P less efficiently affected the infectivity (Figure 4b, chart). Considering that a comparable amount of L274P was expressed (Figure 4b, panels), the data suggest that the DNA binding activity of RAD18 is essential for suppressing HIV-1 infection. To confirm the inhibitory effect of RAD18 domains against HIV-1 replication in human cells, we combined sh-RNA-mediated depletion of RAD18 and complementation of RAD18 wild-type or mutants in 293T cells. Depletion of RAD18 increased

HIV-1 infectivity about twofold, and the complementation of RAD18 wild-type, C28F or D221A reduced the infectivity, but complementation of L274P poorly affected (Figure 4c), which data agrees with the data of Figure 4b. To determine whether RAD18 actually binds to HIV-1 DNA intermediates, we performed immunoprecipitation experiments of RAD18. We transfected 293T cells with myc-tagged RAD18 wild type or L274P, infected with VSV-G pseudotyped HIV-1 24 hours after transfection, harvested 24 hours after infection, and then immunoprecipitated with anti-myc monoclonal antibody. We analyzed virus DNA in the immunoprecipitated RAD18 by quantitative-PCR for HIV-1 LTR sequence. Both RAD18 wild-type and L274P co-precipitated HIV-1 DNA, but L274P did less efficiently than wild-type (Figure 4d). These results strongly suggest that RAD18 inhibit reverse transcription of HIV-1 by directly binding to viral DNA intermediates.

Discussion

We used a set of DNA-repair deficient DT40 cells and identified several candidate DNA repair molecules that might affect HIV-1 infection. Of these, ATM, FEN1, TP53BP1, WRN and RAD18 had been already reported to affect HIV-1; ATM-dependent damage response is stimulated by HIV-1 integrase-induced cleavage of host genomic DNA [24]; FEN1 has been implicated in retroviral DNA integration [12,13]; TP53BP1 was reported to repress mitotic catastrophe in syncytia elicited by the HIV-1 envelope [25]; WRN interacts and cooperates with Tat to transactivate the HIV-1 LTR to promote retroviral replication [26];

RAD18 -/- murine embryonic fibroblasts are more susceptible to HIV-1 infection [16]. Although chicken DT40 cells are not relevant to physiological targets of HIV-1, the screening experiments we performed using DT40 isogenic mutants successfully confirmed the molecules that had been previously reported to affect HIV-1. We, therefore, conclude that our screening data allows for sensitive detection of the host factors that affect the viral life cycle.

We then measured proviral DNA, 2-LTR circles and RT products by quantitative PCR to speculate affected steps of HIV-1 infection. *EXO1 -/-*, *TP53BP1 -/-* and *WRN -/-* cells accumulated lower proviral DNA and higher 2-LTR circles, suggesting that EXO1, TP53BP1 and WRN are associated with the integration step of retroviral infection. EXO1 has been implicated in many different DNA metabolic processes, including homologous recombination [27], which was reported to be associated with the formation of 1-LTR circles in the process of integration [28]. TP53BP1 and WRN have been reported to affect HIV-1 replication [25,26], however, there have been no report of these molecules to be associated with retroviral integration. Since several reports have shown that DSBs repair system were required for retroviral integration, further exploration based on our findings will help reveal how host DNA repair molecules modify retroviral integration.

Mulder et al. first reported that RAD18 interacts with HIV-1 integrase [29], but a follow-up report suggested an integrase-independent mechanism for higher susceptibility to HIV-1 in RAD18 -/- cells [16]. RAD18 is well-known for its function in DNA damage bypass and post-replication repair, where it promotes

monoubiquitylation of PCNA at stalled replication forks. Furthermore, it has been reported that RAD18 is involved in homologous recombination repair [15]. We found increased accumulation of RT products and about twofold higher amount of integrated HIV-1 proviral DNA in RAD18 -/- chicken DT40 cells. These data strongly suggest a reverse-transcription-dependent mechanism for higher susceptibility in these cells. However, no increase of 2-LTR circles in the condition of higher accumulation of RT products does not deny the promotion of integration in these cells. In other words, these data alone cannot directly exclude the integrase-dependent inhibition of HIV-1 by RAD18.

We further explored the mechanism of how RAD18 suppresses HIV-1 infection by utilizing loss-of-function substitutions of RAD18 amino acid residues; C28F, a substitution in the RING domain required for E3 ligase activity and PCNA monoubiquitylation, D221A, a substitution in the zinc finger domain required for homologous recombination, and L274P, a substitution in the SAP domain required for single-strand DNA binding. Our results that only L274P lost HIV-1 suppression demonstrate that DNA-binding is required for RAD18 to suppress HIV-1 infection, but E3 ligase activity or homologous recombination activity is not. We then directly confirmed interactions between RAD18 and HIV-1 DNA by immunoprecipitation experiments followed by quantitative -PCR. We observed the binding of L274P mutant to HIV-DNA was lower than wild-type RAD18, consistent with the previous report that RAD18 L274P mutant is impaired in binding to single-strand DNA [23]. Because RAD18 binds to single-strand DNA, but not to double-strand DNA [23], it is not likely that HIV-1 suppression by RAD18 occurs at the

integration step.

In summary, we have identified several DNA damage response molecules that might support HIV-1 infection. We also confirmed that RAD18 suppresses HIV-1 infection not only in chicken DT40 cells, but also in human 293T and Jurkat cells, and found that direct DNA-binding is critical for this suppression. Our data combined with previous literatures support the model in which RAD18 inhibits reverse transcription by directly binding to viral DNA intermediates, and provide new insights into the mechanism of HIV-1 reverse transcription.

Acknowledgments

We thank the laboratory members for helpful discussion. This work was partly supported by ViiV Healthcare Japan Research Grant to Shindo. This work was partly supported by JSPS Core-to-Core Program.

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Figure legends

Figure 1. Single-cycle infectivity of VSV-G pseudotyped, luciferase-reporter HIV-1 (dark blue bars) and MLV (light blue bars) in DT40 cells deficient in indicated gene. The infectivity of each virus in parental DT40 cells was normalized to 1, and averages and standard errors of three independent experiments are shown. Asterisks indicate statistical significances (*: $P<0.01$, **: $P<0.001$).

Figure 2. Time course of late RT products in VSV-G pseudotyped HIV-1 infected DT40 cells deficient in indicated gene (dotted lines). Data from parental DT40 cells were reproduced in each chart for

comparison (solid lines).

Figure 3. Quantitative-PCR analyses of integrated provirus (orange bars) and 2-LTR circles (blue bars) in DT40 cells deficient in indicated gene. The copy numbers in parental DT40 cells were normalized to 1, and averages and standard errors of two independent experiments are shown. Asterisks indicate statistical significances (*: $P<0.01$, **: $P<0.001$).

Figure 4. DNA binding is responsible for the suppression of HIV-1 by RAD18. (a) HIV-1 infectivity was tested in human 293T cells and Jurkat cells with siRNA-mediated knockdown of RAD18 (top). The efficiency of depletion was confirmed by immunoblotting (bottom). (b) Overexpression of RAD18 wild-type and loss-of function mutants in 293T cells. (left, top) Schematic depicting of functional domains of RAD18 and its loss-of function mutations. (left, bottom) Immunoblotting of the cells used in the infectivity assays. (right) Relative infectivity of HIV-1. Averages and standard deviations of three independent experiments are shown. The infectivity in the absence of RAD18 overexpression was normalized to 1. (c) Knockdown and complementation of RAD18 in 293T cells. (top) Relative infectivity of HIV-1. Average and standard deviations of three independent experiments are shown. The infectivity in the absence of RAD18 overexpression and knockdown was normalized to 1. RAD18 protein levels were analyzed by immunoblotting (bottom). (d) Quantitative-PCR analyses of HIV-1 DNA co-precipitated with

RAD18 (top). Average and standard deviations of three independent experiments are shown. The amount of HIV-1 DNA without RAD18 overexpression was normalized to 1. The amount of precipitated RAD18 protein levels were analyzed by immunoblotting (bottom).

Supplementary Materials

A screening for DNA damage response molecules that affect HIV-1 infection

Noriyoshi Yoshinaga[1], Keisuke Shindo[1], Yusuke Matsui[1], Hirofumi Fukuda[1], Kayoko Nagata[1], Kotaro Shirakawa[1], Masayuki Kobayashi[1], Shunichi Takeda[2] and Akifumi Takaori-Kondo[1]

1) Department of Hematology and Oncology, and 2) Department of Radiation Genetics, Graduate School of Medicine, Kyoto University

Supplementary Table 1. DT40 clones used in this study

Clones	Function of deleted(mutated) gene(s), and annotation	References
LIGASE4	Nonhomologous end-joining	[1]
PRKDC	Nonhomologous end-joining	[2]
TP53BP1	Inhibition of homologous recombination	[3]
ATM	Damage check point control	[4]
RAD9	Damage check point control	[5]
H2AX	Homologous recombination	[6]
RAD54	Homologous recombination	[7]
NBS1	Homologous recombination	[8]
XRCC2	Homologous recombination	[9]
XRCC3	Homologous recombination	[9]
RBBP8	Homologous recombination	[10]
GEN1	Homologous recombination	[11]
UBC13	E2 ligase, post-replication repair, homologous recombination	[12]
RNF8	E3 ligase, DSB repair	[13]
RAD18	E3 ligase of PCNA, post-replication repair	[14]
PARP-1	DNA damage sensing, poly-ADP ribosylation, SSB and DSB repair	[15]
TDP1	Removal of Top1 cleavage complex (Top1cc)	[16]
FANCC	Interstrand crosslink repair, homologous recombination	[17]
SNM1A1B	Interstrand crosslink repair	[18]
PCNA	Translesion synthesis DNA polymerase	[19]
POLN	Translesion synthesis DNA polymerase	[20]

POLH	Translesion synthesis DNA polymerase	[21]
POLZ	Translesion synthesis DNA polymerase	[22]
POLQ	Microhomology-mediated end-joining	[23]
FEN1	5' flap endonuclease, base excision repair, homologous recombination	[24]
EXO1	Mismatch repair	[25]
MSH3	Mismatch repair	[11]
FBH	DNA helicase, phenotype similar to BLM	[26]
WRN	RecQ helicase responsible for Bloom syndrome	[27]
POLH/POLZ	Translesion synthesis DNA polymerase	[28]
TDP1/RBBP8	Removal of Top1 cleavage complex (Top1cc)/ Homologous recombination	[16]
POLQ/POLN	Translesion synthesis DNA polymerase/ Microhomology-mediated end-joining	[23]

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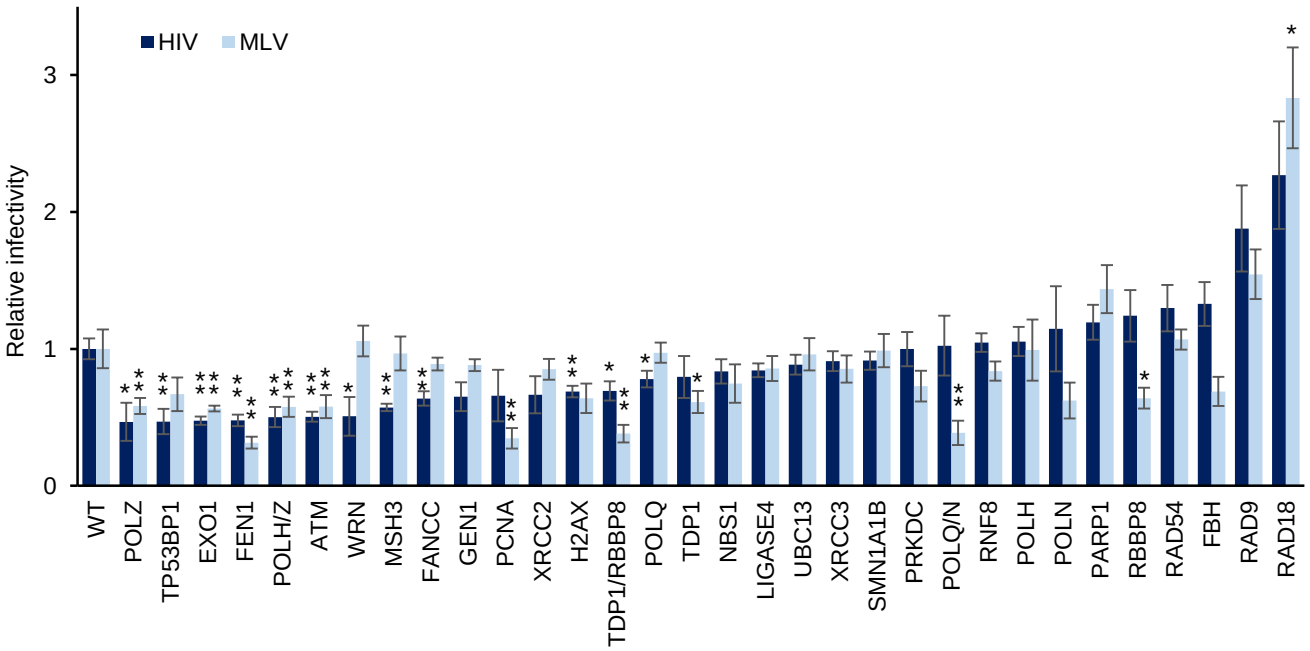
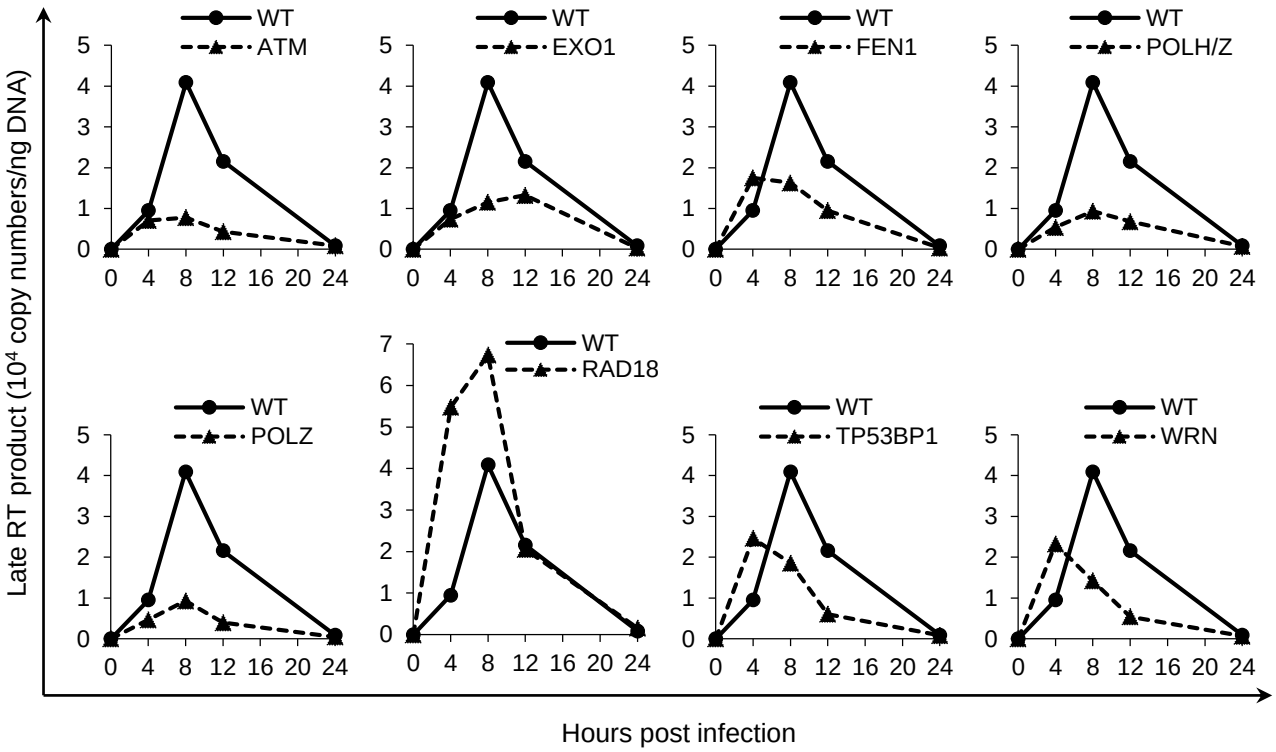
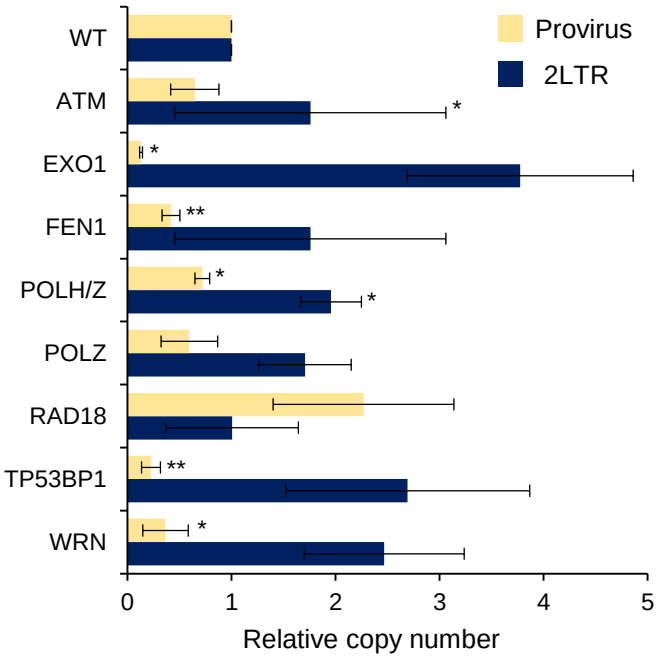
Yoshinaga *et al*., Figure 1



Yoshinaga *et al*., Figure 2



Yoshinaga *et al*., Figure 3



Yoshinaga *et al*., Figure 4