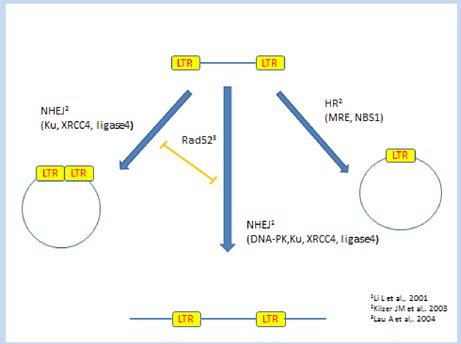
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A screening for DNA repair enzymes that affect HIV-1 infection

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Background

DNA repair enzymes might affect HIV-1 infection, as DNA intermediates of the virus play critical roles in the viral life cycle. Although several such molecules have been reported, interactions between HIV-1 and host DNA repair molecules have not been fully elucidated.



Objectives

· To identify DNA repair molecules that affect HIV-1 infection

· To elucidate the mechanisms how DNA repair molecules are related to HIV-1 infection

Materials and Methods

Cell lines:
To screen for DNA repair enzymes related to HIV-1 replication, we used a set of knockout DT40 cell lines deficient in genes involved in DNA repair pathways.

Q1
Single-cycle infection of luciferase-reporter HIV-1 and MLV in DT40 cells.

Q2
Quantitative PCR analyses of late reverse transcription products, 2-LTR circle DNA, and integrated provirus. A primer for chicken repeat 1 sequence was used for detecting integrated provirus in chicken DT40 cells.

Q3, Q4
Single-cycle infectivity of HIV-1 in human 293T cells over-expressing wild-type or mutant (C28F, D221A and L274P) homo sapiens RAD18.

Q5
For co-immunoprecipitation to test interaction of RAD18 to HIV-1 DNA, 293T cells were transfected with pRAD18-myc or its L274P variant. At 24 hours post-transfection, cells were infected with HIV-1. Cell lysates were prepared at 12 hours post infection, and performed immunoprecipitation of myc–tagged protein. The immunoprecipitates were used for PCR analysis in order to detect HIV DNA.

Q1. Which DNA repair molecules are involved in HIV-1 replication?

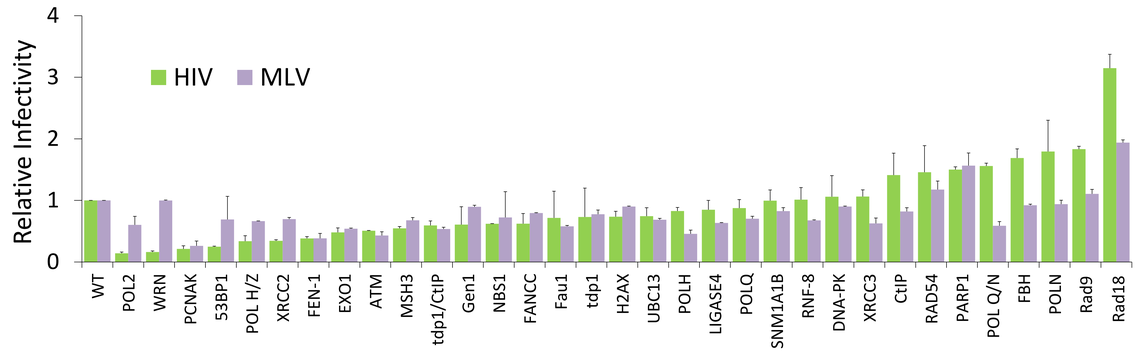


Figure 1. Single-cycle infectivity of luciferase-reporter HIV-1 (*green bars*) and MLV (*gray bars*) in DT40 cells deficient in indicated gene. The infectivity of each virus in wild-type DT40 cells was normalized to 1, and averages and standard errors of two independent experiments are shown.

Q2. Which steps in the viral life cycle of these candidate molecules function?

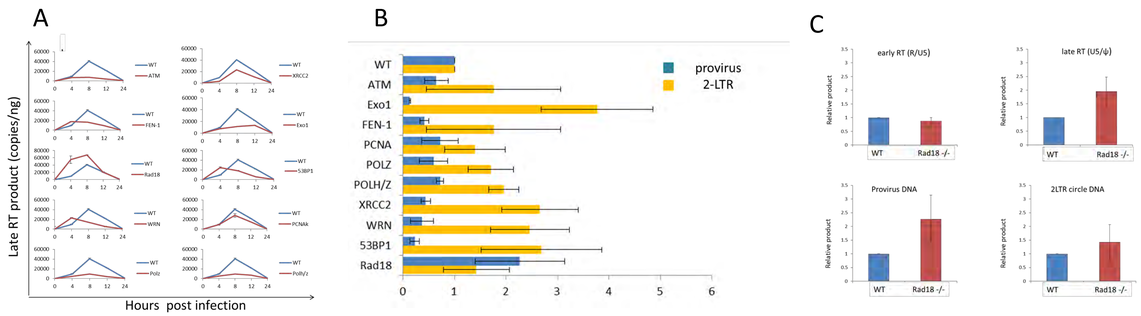


Figure 2. Quantitative-PCR analyses of Late RT products (A), 2-LTR circles (B, yellow bars) , and integrated provirus (B, blue bars) in DT40 cells deficient in indicated gene. Quantitative-PCR analyses of early RT product, Late RT products, 2-LTR circles , and integrated provirus in DT40 cells deficient in Rad18 genes (C). Averages and standard errors of three independent experiments are shown.

Q3. Does human RAD18 restrict HIV-1 infection in human cells?
Q4. Which domain of RAD18 is required for restricting HIV-1 infection?

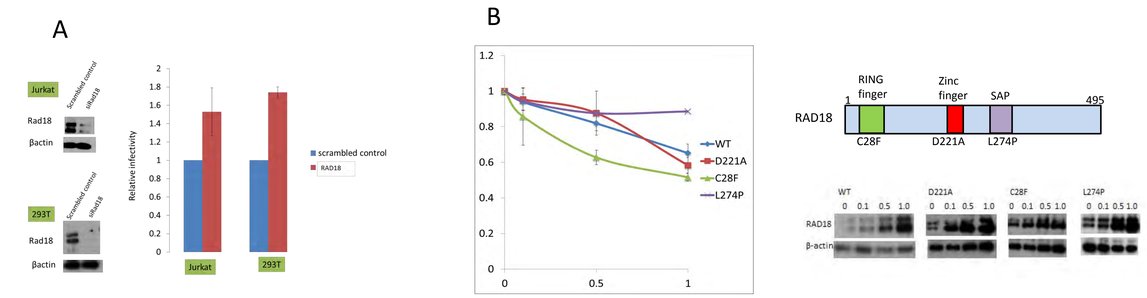


Figure 3. siRNA-mediated knockdown of hRAD18 increased HIV-1 infectivity in Jurkat cells and 293 T cells (A). HIV-1 infectivity of human 293T cells over-expressing wild-type or mutant homo sapiens RAD18 (B).

Q5. Can RAD18 bind to HIV-1 DNA?

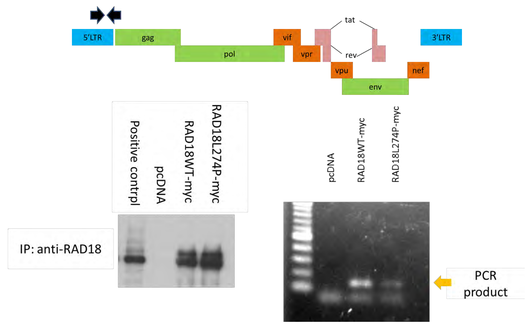


Figure 4. RAD18 WT co-preciptated HIV-1 DNA, and L274 variant less efficiently did than WT.

Discussion

- RAD18 might inhibit reverse transcription by directly binding to viral DNA intermediates (Figure 5).
- Exo1, 53BP1, XRCC2 and WRN are supposed to be required for integration of viral reverse transcription products (Figure 6).

Figure 5

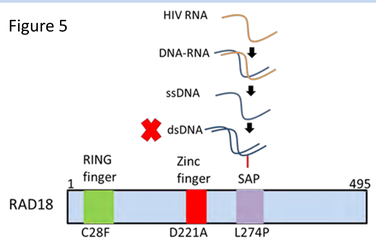
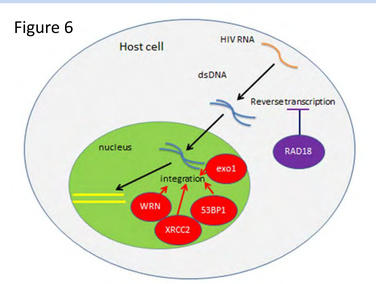


Figure 6



Acknowledgments

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