Potential of Radiation-Induced Cellular Stress for Reactivation of Latent HIV-1 and Killing of Infected Cells

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Abstract

The use of highly active antiretroviral therapy against HIV-1 for last two decades has reduced mortality of patients through extension of nonsymptomatic phase of infection. However, HIV-1 can be preserved in long-lived resting CD4⁺ T cells, which form a viral reservoir in infected individuals, and potentially in macrophages and astrocytes. Reactivation of viral replication is critical since the host immune response in combination with antiretroviral therapy may eradicate the virus (shock and kill strategy). In this opinion piece, we consider potential application of therapeutic doses of irradiation, the well-known and effective stress signal that induces DNA damage and activates cellular stress response, to resolve two problems: activate HIV-1 replication and virion production in persistent reservoirs under cART and deplete infected cells through selective cell killing using DNA damage responses.

HIV-1 CAN BE preserved in long-lived resting CD4⁺ T cells, which form a viral reservoir in infected individuals. This reservoir may persist for many years even though the patients are usually treated with highly active antiretroviral therapy (HAART), and viral population can be recovered once HAART is stopped. Thus, the selective activation of latently HIV-infected cells resulting in the replication of proviral genome is critical to make infected cells recognized by the immune system. The host immune response may eliminate the virus following reactivation (shock and kill strategy). Another important issue is selective activation of apoptosis in the infected cells after reactivation. This might increase the chance of infected cells to be killed without death of uninfected T cells. Additionally, elicitation of the treatment that can directly eliminate latently HIV-infected cells within the pool of resting T cells is also important for effective depletion of viral reservoirs.

The major problem in eradication of HIV from the patients is the lack of therapeutic approaches to recognize the latent HIV-1 provirus and to eliminate latently infected cells. Relatively low frequency of latently infected T cells in patients ($\sim 1/10^5$ – 10^6 in the resting CD4⁺ T-cell population¹) and lack of biomarkers for the latent infection make these cells undetectable for the available therapies. Current ideas to use HDAC inhibitors (disulfiram,² vorinostat³), PKC agonists, or bromodomain inhibitors for virus reactivation are promising since

these latency reactivation agents (LRAs) provide robust increase of viral replication without release of proinflammatory cytokines.^{4,5} However, according to recent *in vivo* studies, treatment with vorinostat, despite transient activation of HIV-1 transcription, does not induce killing of latently infected cells and thus does not reduce the latent reservoir.^{6,7} Therefore, for successful eradication of HIV-1 through selective killing of infected cells within the scope of shock and kill strategy, additional approaches are required.

Ionizing irradiation (IR) is the well-known and effective stress signal that induces DNA damage and activates cellular stress response. The typical X-ray- and γ -IR-induced DNA lesions cause double-strand breaks that can result in induction of up to four independent repair pathways: homologous recombination, nonhomologous end joining, (NHEJ), alternative NHEJ, and single-strand annealing (reviewed in⁸). X-ray IR stress usually induces NHEJ machinery,⁹ which is associated with enhanced activity of many cellular factors involved in transcription activation, such as NF- κ B,¹⁰ Sp1,¹¹ HAT1,¹² and CBP/p300 whose activation has been shown to lead to SWI/SNF-mediated chromatin remodeling.^{13,14}

Earlier studies of the effect of DNA-damaging agents, such as ultraviolet IR, and radiotherapy (X-ray, γ IR) on HIV-1 demonstrated that induced SOS-like stress responses enhanced viral gene expression to levels that are similar to those obtained by the tat gene product^{15–18} and induced cell death

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through chromatin DNA damage.¹⁹ Both X-ray and γ -IR have been shown to activate LTR-driven transcription through the activation of NF- κ B DNA binding.^{16,17} Our earlier studies indicated that single dose of γ -IR differently activated infected T cells compared with the parental uninfected cell lines. Exposition of chronically HIV-1-infected T-cell lines, ACH2 and 8E5, to γ -IR resulted in inappropriate entry of these cells into the S phase and eventual apoptosis, whereas the uninfected parental cells, CEM and A3.01, demonstrated typical response for the transformed cells, namely arrest at G1/S and G2/M phase of cell cycle before repair.²⁰ The irradiated chronically infected T cells also displayed an increased level of HIV-1 mRNA accumulation (both unspliced and spliced) and the viral proteins encoded by the mRNAs, specifically Tat, and Rev, Vpr, and Nef. These data suggested that the nuclear accumulation of HIV-1 proteins, such as Tat and Vpr, may contribute to aberrations in cell cycle regulation, resulting in the loss of G1/S checkpoint.

Later, we demonstrated that despite IR having equally induced p53-mediated DNA repair pathways in HIV-1-infected and uninfected cells (phosphorylation of Ser9 and Ser15 residues^{21–23}), the phosphorylation of Ser46, which is responsible for the ability of p53 to induce apoptosis,²⁴ was approximately higher in the infected T cells. These data may potentially explain the lower viability of primary HIV-1-infected CD4⁺ T cells after IR compared with uninfected cells.^{25,26} Other studies reported that the X-ray-treated human colonic carcinoma cells could activate NF- κ B-mediated HIV-1 transcription in nonirradiated cells through the secretion of cell-activating cytokines,²⁷ indicating an indirect effect of IR on HIV-1-infected cells.

Despite dramatic activation of HIV-1 transcription after the treatment with certain combinations of LRAs,⁵ this treatment alone does not provide effective death of infected cells and depletion of latent reservoir.^{6,7} We recently proposed that IR alone was sufficient to activate the HIV-1 LTR and effectively kill the infected T cells. This killing was especially more pronounced in primary T cells, including latently infected CD4⁺ memory T cells. We found that cytopathogenic effect of IR on reactivated HIV-1-infected primary CD4⁺ T cells was more than twofold higher than on the uninfected cells.²⁸ Recent clinical studies that included analysis of CD4⁺ T-cell count in the blood from cancer patients infected or not with HIV-1 indicated that radiation therapy reduced the CD4⁺ cell count more dramatically in HIV-1-infected individuals.^{25,29} Treatment with NNRTI-based ART exacerbated this effect.²⁶

In addition, another study from Hanauske-Abel and colleagues similarly showed that apoptosis can be preferentially activated in HIV-infected T cells and in peripheral blood mononuclear cells using the topical antifungal ciclopirox and the iron chelator deferiprone, which trigger preferential elimination of infected, but not uninfected, cells.³⁰ No viral reemergence was observed even 12 weeks after drug cessation, suggesting elimination of the proviral reservoir. In our experiments with monocytes/macrophages, IR alone did activate HIV-1 LTR, but did not kill infected cells, which is consistent with previously published data about relative resistance of macrophages to cancer radiotherapy.^{31,32} Nevertheless, the combination of moderate doses of IR with chemical compound, PKC agonist bryostatin 1, enhanced transcription activation and cell killing in monocyte/macro-

phages.²⁸ This suggests that the IR effect is cell-type specific and that infected T cells are more prone to apoptosis with IR, whereas IR with LRAs is needed to kill infected myeloid cells.

Another reason to consider low doses of IR as a potential therapeutic approach against latent HIV-1 infection is an impact of stress-induced apoptosis on HIV-1 replication. A recent publication from Zeichner laboratory indicated that cancer chemotherapeutic agents that induce apoptosis dramatically activated HIV-1 replication in latently infected cells.³³ The authors based their study on the observation that cytotoxic chemotherapy and bone marrow transplantation applied to HIV patients who developed lymphoma and appeared to be relatively more effective at depletion of the HIV-1 latent reservoir than LRAs.^{34,35} In fact, treatment of both persistently HIV-1-infected T cells and monocytes with cytotoxic drugs, doxorubicin, etoposide, fludarabine phosphate, or vincristine, was associated with caspase-3 and -8 activation and therefore apoptosis response that followed dramatic increase of HIV-1 RNA and p24 count.

The only known case of HIV-1 cure in the patient known as Berlin Patient supports the essential role of programmed death of HIV-infected cells for depletion of viral reservoir and, at the same time, applicability of IR as a potential anti-HIV treatment. The IR doses used for total body IR in the therapy of lymphoma (two single doses, 2 and 4 Gy) were applied to this patient before transplantation of the bone marrow with CCR5 Δ 32 mutation and did not have documented negative effect on other tissues.^{34,36} Meanwhile, the virus was likely eradicated from latent reservoirs, including tissues of the central nervous system, since further prolonged tests did not reveal HIV-1 in both blood and spinal fluid specimens,³⁶ whereas two other HIV-1-infected lymphoma patients, called Boston patients, received only chemotherapy before transplantation of wild-type CCR5(+) stem cells.³⁵ Both patients displayed relapse of HIV-1 infection in 6 months after stop of ART.

Another potential application of radiotherapy to treat HIV-1 infection is the use of radiolabeled antibodies targeting viral envelope glycoproteins exposed on the surface of infected cells.

Antibodies to HIV-1 gp120 and gp41 labeled with alpha-emitting radionuclides, ²¹³Bi and ¹⁸⁸Re, were tested on chronically HIV-1-infected human T cells, ACH-2, and infected peripheral blood mononuclears (PBMCs) *in vitro*. Selective killing of infected cells after treatment with both antibodies, including dose-dependent 99% death after ²¹³Bi-anti-gp120, was detected.³⁷ *In vivo* experiments with immunodeficient (SCID) mice intraperitoneally injected with human PBMCs from the spleens and peritoneum and infected with HIV-1 also demonstrated 5- to 10-fold dose-dependent reduction of HIV-1 infection after treatment with ²¹³Bi-anti-gp41.³⁸ Authors declared that this treatment did not cause acute hematologic toxicity in the treated mice. It should be noted here that in spite of demonstrated efficiency of this approach to eliminate productively HIV-1-infected cells, the tested antibodies will not deplete latent reservoir. However, reactivation of latent reservoir before this treatment might potentially increase the level of subsequent depletion of the pool of infected cells.

Our overall view of the HIV-1 transcription and apoptosis of infected cells by IR is depicted in Figure 1. Previous multiple manuscripts have shown that Tat regulates p53

FIG. 1. Effect of irradiation (IR) on infected versus uninfected cells. The diagram is a depiction of what may be occurring in HIV-1-infected T cells versus uninfected cells. Infected cells exposed to low-level IR (either single or multiple low doses) would deregulate the p53 pathway (i.e. p53/Tat binding^{39,40,49,50}) and allow cells inappropriately to enter the S phase. The cells would then either enter apoptosis at the S or move to the G₂ phase, followed by enhanced apoptosis through Vpr. Vpr has been shown to regulate the DNA damage response.^{45–47} The net effect is apoptosis of infected cells with increased viral release. Other potential deregulation of DNA damage response has been observed in HIV-infected cells through the DNA-PK/p53 pathway^{51–57} and other retroviruses, including HTLV.^{58–62} On the other hand, uninfected T cells, when exposed to IR, would activate their normal p53, leading to activation of p21/waf1 and binding to cyclin/cdk complexes (i.e., cdk2/E, cdk2/A, or cdc2/B), resulting in blocks at the G₁/S or G₂/M for DNA repair. After a certain period of time required for DNA repair, cells would resume into the next phase of replication. The model assumes that viral gene expression is increased with IR over time and cellular apoptosis is enhanced by irreversible DNA damage to the infected cells, while the virus is replicating.

function and that it can sequester p53's effect on p21/waf1 activation.^{39,40} This would require certain levels of HIV-1 basal transcription and protein synthesis related to viral open reading frames (i.e., inactive methylated Tat;^{41–43}) potentially present in some latent cells. Whether latent T cells *in vivo* have actual full-length transcripts, followed by translation of doubly spliced messages such as Tat in the presence of antiretrovirals, has never been formally shown; however, it is equally difficult to obtain pure latent infected cells for quantitative RT-PCR and almost impossible with current technologies to detect low-level proteins such as Tat in these samples. Therefore, the theoretical assumption is that p53 is deregulated by viral proteins such as Tat, which allows cells to progress inappropriately into the S phase with IR activation.

Accumulation of cells at the S phase and lack of appreciable DNA repair would cause apoptosis from the S phase. This also follows an assumption that latently infected cells may not be at the G₀ phase of cell cycle as no publications to date describe the difference between G₀ or early G₁ phase in latently HIV-1-infected cells under cART. We presume that cells may be blocked at the early, mid, or late G₁ phase with the help of viral noncoding RNAs, such as free TAR RNA.⁴⁴ We recently have shown that TAR is present in cART-treated cells where it activates the TLR3 pathway and deregulates protein kinase R innate immune response, which could presumably keep cells away from G₀ and be maintained at early G₁ phase. Alternatively, if cells do pass into the G₂ phase,

there is high translation of viral proteins such as Vpr, which not only blocks cells at G₂/M but also further activates DNA damage response.^{45–47} This would push cells into the G₂ phase to have increased viral protein synthesis and ultimately high viral expression, translation, packaging, and apoptosis of the host cell.

Two other related issues, which can equally contribute to apoptosis or lack thereof, include (1) latent cells may already have increased proteins related to DNA damage machinery as a result of viral integration or leaky LTR read through that is not translated (i.e., short TAR RNA), which could contribute to altered DNA damage response and sensitivity to IR⁴⁸; (2) myeloid cells that are inherently differentiated are locked at the early G₁ phase (2N) and the IR can increase gene expression, but would have minimal to no effect toward apoptosis of the infected cells at the S or G₂/M, making the therapeutic value of IR alone for apoptosis in these cells not significant. Under these conditions, there may need to be a second agent that pushes infected myeloid cells (inherently more resistant to apoptosis compared with uninfected cells) into the apoptotic pathway (i.e., p53-independent pathway).

On the other hand, uninfected cells can tolerate low levels of IR by activating its wild-type p53 and p21/waf1 induction, which would bind to all G₁/S cyclin/cdk complexes and inhibit their Rb phosphorylation. This would block cells transiently at the G₁/S or the G₂/M and allow enough time to repair the damaged DNA, followed by release of cells to move forward into the next stage of replication or simply stay

quiescent after few passages. Again, whether all forms of T cells are equally sensitive to low level of IR remains to be determined as no such data exist to date (i.e., differences between central memory [TCM] vs. transitional memory [TTM] T cells). In conclusion, the potential for IR activation of the HIV promoter with subsequent apoptosis of infected cells offers an attractive mode of shock and kill; however, for the approach to be fully realized in either AIDS patients under cART or AIDS patients with cancer, it requires not only infectious disease expertise but also a basic understanding of radiation oncology. Along these lines, lessons learned from cancer radiation oncology, which has proven to be effective for more than five decades when using low-level IR treatment, make the approach a manageable solution in immunocompromised patients.

Acknowledgments

The authors would like to thank the members of the Kashanchi Laboratory, especially Angela Schwab, for assistance with the manuscript and Figure 1. This work was supported by NIH grants, AI043894, AI114490, and AI113140, to F.K. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Disclosure Statement

No competing financial interests exist.

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