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HIV eradication: Targeting viral reservoir cells and replenishing with HIV resistant cells

Jeanne Adiwinata Pawitan
Department of Histology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba 6, Jakarta, (INDONESIA)
E-mail : jeanneadiwip@fk.ui.ac.id; jeanneadiwip@gmail.com

ABSTRACT

Latent HIV infection needs a life-long antiretroviral treatment, as there is no means to completely eradicate the virus. Therefore, to manage this condition, new approaches should be developed. This article highlighted the results of molecular and proteomic studies that can be used in identifying viral reservoir cells to destroy viral shelter, which might lead to complete viral eradication. Further, administration of engineered HIV resistant autologous hematopoietic stem cells will prevent subsequent infection by exacerbating reminiscent HIV virus. However, problems and limitations in the technology to provide resistant cells still needs to be solved. © 2013 Trade Science Inc. - INDIA

KEYWORDS

CCR5;
HIV;
Proteome;
Reservoir cells;
Surface protein;
Zn finger nuclease.

INTRODUCTION

Antiretroviral therapy of HIV may reduce viral load and stop the progress of disease in most patient. Treated patients will develop latent infection due to provirus integration to host genome. Provirus integration occurs in latently infected cells that become a reservoir, which provide a shelter to the virus. Therefore, a life-long treatment with antiretroviral is required, as until to date there is no means to completely eradicate the virus[1,2].

Targeting viral-reservoir cells may destroy the shelter and thus leads to complete viral eradication. The problem is that the latently infected cells are difficult to be distinguished from uninfected cells[2]. Therefore, means to identify the latently infected cells is crucial to target and destroy these cells. Further, destroyed infected cells should be replaced by engineered HIV virus resistant cells. However, problems and limitations in the technology to provide resistant cells still needs to be solved.

MEANS TO IDENTIFY LATENTLY INFECTED CELL

Various proteomic studies on HIV infected cells compared to uninfected cells have been published [3-5]. A study on HIV-1 infected human T lymphocyte cell lines (PM1) revealed changes in protein expressions (proteome) over time i.e. 42 hours after a massive amount of viral inoculation, and 7-10 days after inoculation. Proteome changes at 42 hours after a massive amount of viral inoculation were used to detect acute infection changes, which were the results of a single round of replication. Further, proteome changes around 7-10 days after inoculation represent changes at the peak of infection, and were used to detect long term

effect of viral replication. Proteome analysis at the peak of infection showed 65 upregulated proteins, while after a single round of replication, only 8 proteins were upregulated. Moreover, the HIV capsid protein (CA-p24) and HIV accessory protein (Nef) were upregulated in both[3].

Another proteomic study on chronic HIV-infection of T cells showed the exclusive expression of a membrane receptor protein tyrosine kinase (PTK) namely Zeta chain tyrosine-protein kinase (ZAP-70) on the plasma membrane of infected T cells. This PTK is associated with the zeta chain of T cell receptor (TCR)[4].

Further, a proteomic study on peripheral blood mononuclear cells (PBMCs) that compared PBMCs of HIV-positive patients to those of healthy controls showed 12 upregulated proteins in HIV-positive patients. This finding was confirmed by mRNA expression that was analyzed by real time RT-PCR. The upregulated proteins were cellular proteins, such as vinculin, filamin-A, ENO1, and L-lactate dehydrogenase B chain (LDHB), which were related to viral proteins to form compound viral-host proteins e.g. vinculin-nef, filamin A-pol, ENO1-rev, and LDHB - gp41 or gp120. In addition, there were other significantly increased cellular surface proteins (e.g. talin) that may be regarded as specific infected-cell proteins[5].

Reservoir cell identification might be possible by using cell surface proteins that are expressed exclusively in HIV infected cells, particularly in latently infected cells. Therefore, knowledge of surface proteins that are expressed exclusively in those cells is needed. For this purpose, revealing compound viral–host proteins by proteomic studies on HIV-latency cell model is crucial to develop a tool for the identification of latently-infected cells.

A study has developed a stable in vitro model for HIV-latency in T cells that can be activated using doxycycline[2]. Proteomic studies on these cells may reveal compound viral-host protein on the surface of latently HIV infected cells, which may be used to eradicate the reservoir cells.

To eradicate the reservoir cells, drugs (toxins) that only kill the latently infected cells may be used. To avoid destruction of normal cells the toxin can be linked to a specific antibody against latently infected cell surface protein, so that the toxin only targets the viral reservoir and spare normal cells. Mutant Pseudomonas exotoxin PE38QQR (PE) that was used to target glioma cells[6], or other toxins may be used for this purpose.

REPLACEMENT OF ERADICATED RESERVOIRS BY HIV RESISTANT CELLS

After infected cell eradication, administration of engineered HIV resistant autologous stem cells is needed to replace the eradicated cells. This approach is to prevent subsequent infection by exacerbating reminiscent HIV virus, which may lead to a final cure for HIV patients in the future. This point of view is supported by a long term study[8-10], which reported the cure of an HIV patient with acute myeloid leukemia who received allotransplantation of hematopoietic stem cells from an HIV resistant donor. The patient is viral-free without anti retroviral therapy since more than 3.5 years[10], and the patient's blood cells were replaced by HIV resistant blood cells[8-10].

The donor was homozygous for a 32 base pair deletion of the CCR5 gene that causes a frame shift and a premature stop codon, which renders the CCR5 to be inactive. Such deletion does not cause any harmful effect on the donor health. CCR5 is a receptor on CD4 cells that is required for CCR5 trophic (R5 type) HIV-1 viral entry. Therefore, inactive CCR5 prevents further viral entry[8-10].

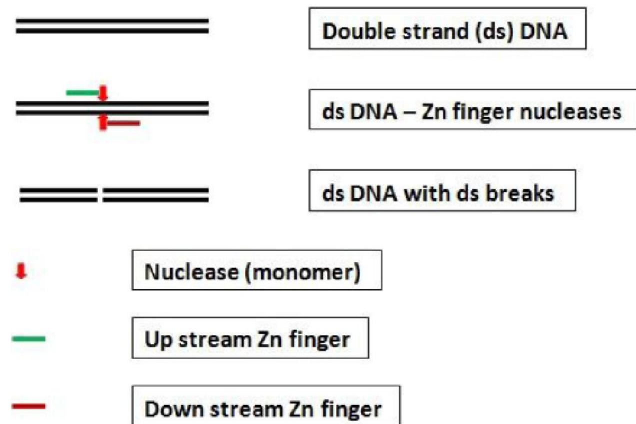
The success of HIV resistant hematopoietic stem cell allotransplantation was considered as a premature conclusion[11], and as the transplantation was done on a case of malignancy that received hematopoietic cell ablation therapy, there is a possibility that the ablation therapy contributed in the elimination of viral reservoirs. Therefore, combination of viral reservoir targeting and HIV-resistant stem cell therapy may become an interesting approach in the future[12]. However, to find a matching HIV resistant donor may pose a problem[12]. This problem can be overcome by engineering HIV resistant stem cells using patient-derived cells.

Methods to induce patient-derived adult cells into induced pluripotent stem cells (iPSCs) are available; and further the iPSCs can be differentiated into desired cells[13]. The desired patient-derived cells, which are the target of HIV can be used to engineer HIV resistant target cells. Perez et al (2008) has engineered HIV re-

sistant CD4 cells using Zinc (Zn) finger nuclease genome editing to disrupt the CCR5 gene. A Zn finger nuclease is a nuclease that is linked to a Zn finger peptide. The Zn finger peptide can be engineered to bind to a particular DNA sequence in a highly specific manner. The nuclease needs to form a dimer to be active. Therefore, a pair of Zn finger nuclease monomers are needed, one with a Zn finger that binds the upstream sequence and another that binds the downstream sequence of the target site. Binding of a pair of Zn finger nucleases causes a double strand DNA break at a specific site (Figure 1). The final result is disruption of the gene due to addition or deletion of nucleotides during DNA repairs[14].

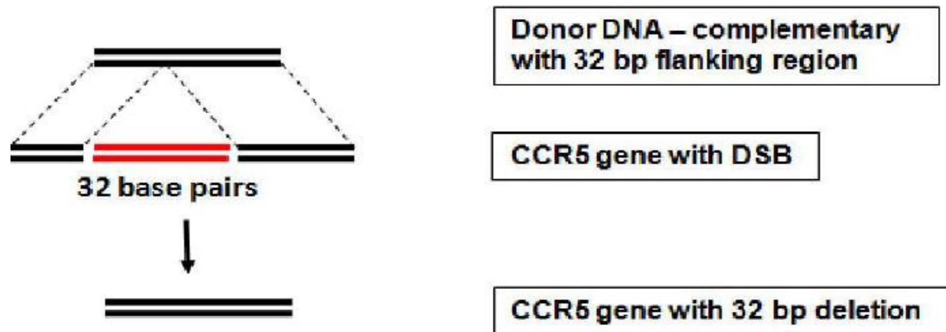
Further, Holt et al (2010) engineered CCR5 knock out human cord blood derived hematopoietic stem cells using the same method, and succeeded in causing disruption in CCR5 gene. Further, the stem cells were transplanted in immune-deficient (NSG) mice. In the NSG mice, the CCR5 knocked out hematopoietic stem cells were able to engraft, proliferate and differentiate into the needed blood cells, including CD4 cells. Further, challenge with HIV virus showed that CCR5 knocked out stem cell transplanted mice had significantly lower HIV level compared to untransplanted mice[15].

Another approach is to engineer hematopoietic stem cells that harbor homozygous 32 base pair deletion in the CCR5 gene, precisely the deletion that occured in naturally resistant individuals. This deletion can be accomplished by using 2 pairs of Zn finger nucleases. The two pairs of nucleases cause double strand DNA breaks at two sites, precisely at the beginning and the end of the 32 base pairs to be deleted (Figure 2-middle). Further, addition of a donor DNA, which is complementary with the 32 base pair flanking region (Figure 2-top), causes a repair with a 32 base pair deletion as the final result (Figure 2-bottom)[16].



ds= double strand

Figure 1 : Generation of a double strand break by using a pair of Zn finger nucleases



bp= base pair, DSB= double strand breaks

Figure 2 : Generation of a deletion by causing 2 double strand breaks

PROBLEMS AND LIMITATIONS OF GENOME EDITING TECHNOLOGY IN PROVIDING HIV RESISTANT CELLS

In the study of Holt et al (2010), cord blood derived hematopoietic stem cells were used to engineer HIV resistant cells, as cord blood is rich in hematopoietic stem cells[15]. However, the use of cord blood will pose rejection problems, and the recipient should be chosen carefully to match the donor. The need to match the recipient to the donor leads to restriction in the use of the engineered cells. Therefore, the use of autologous adipose tissue or other easily accessible tissue derived hematopoietic stem cells using induced pluripotent stem cell method[13,17] may resolve the problem.

Replacement of eradicated reservoirs by engineered HIV resistant cells involves genome editing technolo-

gies. Though this new technology showed promising results, the CCR5 disruption efficiency was still low, i.e. 17±10% of the total CCR5 alleles, and only 5-7% that were homozygous[15].

Moreover, to introduce the Zn finger nucleases into the cells, various vectors can be used, including viral and non viral vectors. Non viral vectors are more favored as they may not give unexpected side effects. Non viral vectors include Zn finger nuclease expressing plasmid. The plasmid was successfully introduced into cells using nucleofection technique. Though in a previous study, this technique was showed to be toxic to human CD34 hematopoietic stem cells and lead to loss of engraftment potential[18], a study that used different parameters of nucleofection succeeded to get a better result[15].

Moreover, Zn finger nuclease genome editing may cause off target double strand break due to homo-dimerization, which may cause undesired disruption on other genes, and thus cytotoxicity. Homo-dimerization occurs at places where there are two identical sequences at both upstream and downstream of a particular site[19]. Therefore, genome wide putative off target screening should be performed, to ascertain that there are no off target disruption.

Recently, to reduce off target disruption, a special nuclease that can not form a homo-dimer was developed. This method showed that the obligate heterodimer greatly reduce the off target disruption[20,21]. Moreover, transcription activator-like effector (TALE) nucleases, which have the same mode of action with Zn finger nuclease have been developed. TALE nucleases that are less toxic to cells compared to Zn finger nucleases[16] can also be used.

CONCLUSION

Targeting viral reservoir cells and administration of autologous HIV- resistant hematopoietic stem cells seems promising in providing a final cure for HIV patient.

REFERENCES

[1] M.I.Johnston, A.S.Fauci; NEJM, **359**, 888 **(2008)**.

[2] R.E.Jeeninga, E.M.Westerhout, M.L.Van Gerven, B.Berkhout; Retrovirology, **5**, 37 **(2008)**.

[3] J.Ringrose, R.E.Jeeninga, B.Berkhout, D.Speijer; J.Virol, **82**, 4320 **(2008)**.

[4] S.Rasheed, J.S.Yan, A.Hussain, B.Lai; J.Transl.Med., **7**, 75 **(2009).**

[5] L.Zhang, X.Jia, X.Zhang, J.Sun, X.Peng, T.Qi, F.Ma, L.Yin, Y.Yao, C.Qiu, H.Lu; Proteome Sci., **8**, 12 **(2010)**.

[6] G.D.King, J.F.Curtin, M.Candolfi, K.Kroeger, P.R.Lowenstein, M.G.Castro; Curr.Gene.Ther., **5**, 535 **(2005)**.

[7] G.Hutter, D.Nowak, M.Mossner, S.Ganepola, A.Musig, K.Allers, T.Schneider, J.Hofmann, C.Kucherer, O.Blau, I.W.Blau, W.K.Hofmann, E.Thiel; N.Engl.J.Med., **360**, 692 **(2009)**.

[8] G.Hütter, T.Schneider, E.Thiel; J.Int.AIDS Soc., **12**, 10 **(2009)**.

[9] G.Hütter, S.Ganepola; ScientificWorld Journal, **11**, 1068 **(2011)**.

[10] K.Allers, G.Hütter, J.Hofmann, C.Loddenkemper, K.Rieger, E.Thiel, T.Schneider; Blood, **117**, 2791 **(2011)**.

[11] J.A.Levy; N.Engl.J.Med., **360**, 724 **(2009)**.

[12] R.Parker, I.Sereti; Blood, **117**, 2746 **(2011)**.

[13] J.Adiwinata Pawitan; Stem Cell International, **2012**, 1 **(2012)**.

[14] E.E.Perez, J.Wang, J.C.Miller, Y.Jouvenot, K.A.Kim, O.Liu, N.Wang, G.Lee, V.V.Bartsevich, Y.L.Lee, D.Y.Guschin, I.Rupniewski, A.J.Waite, C.Carpenito, R.G.Carroll, J.S.Orange, F.D.Urnov, E.J.Rebar, D.Ando, P.D.Gregory, J.L.Riley, M.C.Holmes, C.H.June; Nat Biotechnol, **26**, 808 **(2008)**.

[15] N.Holt, J.Wang, K.Kim, G.Friedman, X.Wang, V.Taupin, G.M.Crooks, D.B.Kohn, P.D.Gregory, M.C.Holmes, P.M.Cannon; Nat Biotechnol, **28**, 839 **(2010)**.

[16] C.Mussolino, R.Morbitzer, F.Lütge, N.Dannemann, T.Lahaye, T.Cathomen; Nucleic Acids Res., **39**, 9283 **(2011)**.

[17] H.C.Kang; Korean.J.Pediatr., **53**, 786 **(2010)**.

[18] R.P.Hollis, S.J.Nightingale, X.Wang, K.A.Pepper, X.J.Yu, L.Barsky, G.M.Crooks, D.B.Kohn; Exp. Hematol., **10**, 1333 **(2006)**.

[19] F.Soldner, J.Laganière, A.W.Cheng, D.Hockemeyer, Q.Gao, R.Alagappan, V.Khurana, L.I.Golbe, R.H.Myers, S.Lindquist, L.Zhang, D.Guschin, L.K.Fong, B.J.Vu, X.Meng, F.D.Urnov, E.J.Rebar, P.D.Gregory, H.S.Zhang, R.Jaenisch; Cell, **146**, 318 **(2011)**.

[20] S.Ramalingam, K.Kandavelou, R.Rajenderan, S.Chandrasegaran; J.Mol.Biol., **405**, 630 **(2011)**.

[21] Y.Doyon, T.D.Vo, M.C.Mendel, S.G.Greenberg, J.Wang, D.F.Xia, J.C.Miller, F.D.Urnov, P.D.Gregory, M.C.Holmes; Nat.Methods, **8**, 74 **(2011)**.