CMI

www.nature.com/cmi

COMMENT

Cell membrane anchoring strategies for HIV gene therapy

Yani Gong[1,2] and Yuxian He[1,2]✉

Cellular & Molecular Immunology (2023) 20:683–685; https://doi.org/10.1038/s41423-023-01006-z

Very recently, the Düsseldorf University Hospital in Germany reported the third patient who was cured of HIV after CCR5Δ32 hematopoietic stem cell transplantation (HSCT) [1]. There is increasing evidence that a cure can be achieved through infusion of HIV-resistant cells for gene therapy. Toward this goal, we focus on cell membrane anchoring strategies by glycosylphosphatidylinositol (GPI), as illustrated in Fig. 1. For example, genetically anchoring the single-domain antibody m36.4 (nanobody) that targets the coreceptor-binding site of gp120 through the GPI attachment signal might render modified cells fully resistant to HIV infection, block HIV-1 envelope-mediated cell–cell fusion and cell–cell viral transmission, and interfere with viral genesis [2].

HIV has been a global public health concern since it was discovered in 1981. The development of effective anti-HIV therapy is a top priority, especially as there is no vaccine available. HIV can integrate into the human genome to form a persistent HIV reservoir that cannot be eradicated by current approaches. Although implementation of highly active antiretroviral therapy (HAART), the so-called "cocktail" therapy, has rendered HIV/AIDS a manageable chronic disease, HAART is lifelong treatment and is associated with drug resistance, toxic damage to vital organs, and high costs. Once HAART is stopped, HIV rebound occurs from the reservoir in the host. Although little is known about the biology of HIV reservoirs, there have been an increasing number of cured or near-cured patients in recent years, especially with regard to posttreatment interruption without a viral rebound for a long time, which is called posttreatment control (PTC) or functional cure. Thus, a functional HIV cure has become the mainstay of current treatment approaches.

"Berlin patient", "London patient" and "Düsseldorf patient" indicate the importance of CCR5Δ32 for HIV remission and that infusion of HIV-resistant cells would be a viable treatment strategy for effecting an HIV cure. Nevertheless, due to the difficulty in finding donors with CCR5Δ32 mutation, the risk of surgery, and the fact that CCR5Δ32 HSCT does not always cure HIV, this HSCT approach is aimed at a very limited population. For safety reasons, membrane-anchored HIV entry inhibitors, such as broadly neutralizing antibodies (bNAbs) and membrane fusion inhibitor peptides, have significant advantages in generating HIV-resistant cells because they block the first step of viral infection, i.e., cell entry.

Initially, Zhou et al. found that linking the single-chain variable fragment (scFv) of the nonneutralizing anti-HIV antibody TG15 to the transmembrane domain of the type I interferon receptor rendered the modified cells resistant to HIV entry [3]. Considering that GPI can covalently anchor many proteins to lipid rafts of the cell plasma membrane for biological functions and that both HIV entry and budding occur in lipid rafts, they characterized different anti-HIV bNAbs as GPI-anchored inhibitors and found that the human antibody X5, specific for the gp120 coreceptor-binding site, to be most effective [4]. Because certain antibodies have an exceptionally long CDR H3 that can form a unique stable subdomain to ensure specificity, Zhou's group further generated a GPI-anchored peptide of CDR H3 derived from bNAb PG16, which neutralized diverse HIV-1 isolates with remarkable potency when expressed on the surface of human CD4 T cells [5]. As antibodies always preferentially bind to assembled viral spines, enrichment of GPI-CDR H3 by forming a trimeric form can result in stoichiometric recognition of 3 or 2 HCDR3 molecules against 1 viral peak, thereby enhancing antiviral activity [6]. Moreover, this group worked on two heavy chain-only llama antibodies, JM2 and JM4. GPI-VHH JM4-modified cells, but not JM2-modified cells, effectively neutralized cell-free and cell-associated HIV infections, revealing that epitope specificity largely determines the function of GPI-anchored anti-HIV antibodies in different forms [7]. As mentioned above, our studies verified GPI-m36.4 as a powerful strategy with multiple anti-HIV mechanisms [2]. Its effect on virus genesis suggests that even if the virus escapes inhibition at the entry step, its replication will eventually be controlled. Misra et al. reported that GPI-scFv can inhibit Env processing and function, thus limiting newly synthesized virus production [8]. In general, discovery of this mechanism provides strong support for the use of GPI-scFv as a potential tool for HIV gene therapy.

Another class of inhibitors that block HIV entry is membrane fusion-inhibitory peptides, as exemplified by T20 (enfuvirtide) derived from the C-terminal heptad repeat (CHR) of gp41. T20 was clinically approved by the U.S. FDA in 2003 as the first entry inhibitor for HIV-1 treatment; however, it has obvious disadvantages, such as requiring a large dosage due to its short half-life and a relatively low genetic barrier for inducing drug resistance. Dorothee von Laer et al. constructed HIV-resistant cells by expressing T20 or C46 on the surface of HIV-susceptible cells via the membrane-spanning domain (MSD) of low-affinity nerve growth factor receptor (LNGFR) [9]. The C34 peptide is also a more potent fusion inhibitor than T20. Leslie et al. conjugated C34 to the HIV coreceptors CCR5 and CXCR4, demonstrating that C34-CXCR4 is the most efficient approach, and infusion of C34-CXCR4-modified CD4 T cells is currently under clinical trial [10]. With the GPI anchoring strategy, Liu et al. expressed the C34 fusion gene on the cell surface and achieved a maximum inhibition percentage of transduced cells comparable to that of a very high concentration of soluble C34 [11]. In our study, we constructed GPI-2P23, which mainly targets the conserved gp41 pocket site with an M-T hook structure [12]; as expected, it was highly effective against T20- and

[1]NHC Key Laboratory of Systems Biology of Pathogens, Institute of Pathogen Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, 100730 Beijing, China. [2]Center for AIDS Research, Chinese Academy of Medical Sciences and Peking Union Medical College, 100730 Beijing, China. ✉email: yhe@ipb.pumc.edu.cn

Received: 11 March 2023 Accepted: 15 March 2023
Published online: 27 March 2023

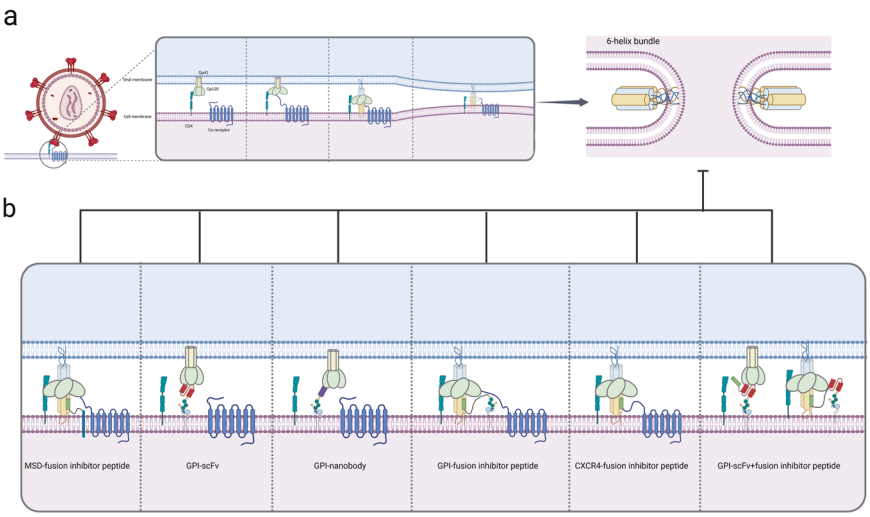

Fig. 1 Generation of HIV-resistant cells through membrane-anchored HIV entry inhibitors. **a** Process of HIV-host cell membrane fusion mediated by 6-helix bundles (6-HB). **b** Expression of HIV entry inhibitors on the surface of target cells utilizing membrane anchoring can effectively prevent the occurrence of membrane fusion. Several membrane anchoring approaches, including the membrane-spanning domain (MSD) of the low-affinity nerve growth factor receptor (LNGFR), glycosylphosphatidylinositol (GPI) attachment signal, and HIV coreceptor CXCR4, are illustrated. HIV entry inhibitors are broadly neutralizing antibodies (bNAbs) and fusion inhibitor peptides. scFv single-chain variable fragment

C34-resistant mutants. Recently, Mazurov et al. embedded a series of short fusion inhibitor peptides into the shortest GPI-anchored protein CD52 [13]. CEM/R5 cells with double knock-in of MT-C34 and 2P23 at the CXCR4 locus were obtained through CRISPR/Cas9 technology, showing a higher level of protection compared to single knock-in cells. Moreover, the GPI-anchored peptide derived from CD52, combined with improved nuclear import of enhanced donor vectors and CRISPR/Cas9 technology, improved knock-in efficiency in primary cells [13]. Considering the high variability of HIV and the susceptibility to escape mutant viruses, we recently developed a panel of bifunctional GPI-anchored inhibitors by linking the fusion-inhibitory peptide P41 and the scFv of bNAb 10E8, which showed very potent and broad-spectrum anti-HIV activity with a potential high genetic resistance barrier [14].

Given the persistence of a complex, diffuse, and diverse HIV reservoir, many efforts have focused on large-scale sequencing analyses of reservoir cells [15]. However, to date, the composition and distribution of the reservoirs have not been elucidated; instead, the data have only demonstrated that HIV shows diversity in each patient and that reservoir distribution tends to be random and lacks absolute regularity. Because GPI-anchored entry inhibitors not only protect modified cells from viral invasion and confer a stronger survival advantage over unmodified cells but also reduce generation of daughter viruses as well as their infectivity, the resulting HIV-resistant cells in combination with immunotherapy might lead to a functional cure or even eradication. Further studies are needed to address how GPI-anchored antibodies or fusion inhibitor peptides exert viral suppressive effects, the in vivo therapeutic effects and potential side effects as genetic interventions before they can be evaluated in a clinical setting. As a gene therapy approach, a great challenge for GPI anchoring strategies to achieve a meaningful functional cure is in the persistence of the modified cells, taking into account the immunogenicity and cytotoxicity of the transgene, optimal receptor conditions, cell dose, etc., and whether it is mutually exclusive with other therapies requires considerable in vivo studies and clinical trials.

REFERENCES

1. Jensen BO, Knops E, Cords L, Lübke N, Salgado M, Busman-Sahay K, et al. In-depth virological and immunological characterization of HIV-1 cure after CCR5Delta32/Delta32 allogeneic hematopoietic stem cell transplantation. Nat Med. 2023;29:583–87.
2. Jin H, Tang X, Li L, Chen Y, Zhu Y, Chong H, et al. Generation of HIV-resistant cells with a single-domain antibody: implications for HIV-1 gene therapy. Cell Mol Immunol. 2021;18:660–74.
3. Lee SJ, Garza L, Yao J, Notkins AL, Zhou P. A nonneutralizing anti-HIV-1 antibody turns into a neutralizing antibody when expressed on the surface of HIV-1-susceptible cells: a new way to fight HIV. J Immunol. 2004;173:4618–26.
4. Wen M, Arora R, Wang H, Liu L, Kimata JT, Zhou P. GPI-anchored single chain Fv-an effective way to capture transiently-exposed neutralization epitopes on HIV-1 envelope spike. Retrovirology. 2010;7:79.
5. Liu L, Wen M, Wang W, Wang S, Yang L, Liu Y, et al. Potent and broad anti-HIV-1 activity exhibited by a glycosyl-phosphatidylinositol-anchored peptide derived from the CDR H3 of broadly neutralizing antibody PG16. J Virol. 2011;85:8467–76.
6. Liu L, Wang W, Yang L, Ren H, Kimata JT, Zhou P. Trimeric glycosylphosphatidylinositol-anchored HCDR3 of broadly neutralizing antibody PG16 is a potent HIV-1 entry inhibitor. J Virol. 2013;87:1899–905.
7. Liu L, Wang W, Matz J, Ye C, Bracq L, Delon J, et al. The glycosylphosphatidylinositol-anchored variable region of llama heavy chain-only antibody JM4 efficiently blocks both cell-free and T cell-T cell transmission of human immunodeficiency virus type 1. J Virol. 2016;90:10642–59.
8. Misra A, Gleeson E, Wang W, Ye C, Zhou P, Kimata JT. Glycosyl-phosphatidylinositol-anchored anti-HIV Env single chain variable fragments interfere with HIV-1 Env processing and viral infectivity. J Virol. 2018;92:e02080–17.
9. Egelhofer M, Brandenburg G, Martinius H, Schult-Dietrich P, Melikyan G, Kunert R, et al. Inhibition of human immunodeficiency virus type 1 entry in cells expressing gp41-derived peptides. J Virol. 2004;78:568–75.
10. Leslie GJ, Wang J, Richardson MW, Haggarty BS, Hua KL, Duong J, et al. Potent and broad inhibition of HIV-1 by a peptide from the gp41 heptad repeat-2 domain conjugated to the CXCR4 amino terminus. PLoS Pathog. 2016;12:e1005983.
11. Liu L, Wen M, Zhu Q, Kimata JT, Zhou P. Glycosyl phosphatidylinositol-anchored C34 peptide derived from human immunodeficiency virus type 1 Gp41 is a potent entry inhibitor. J Neuroimmune Pharmacol. 2016;11:601–10.
12. Tang X, Jin H, Chen Y, Li L, Zhu Y, Chong H, et al. A membrane-anchored short-peptide fusion inhibitor fully protects target cells from infections of human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus. J Virol. 2019;93:e01177–19.
13. Maslennikova A, Kruglova N, Kalinichenko S, Komkov D, Shepelev M, Golubev D, et al. Engineering T-cell resistance to HIV-1 infection via knock-in of peptides from the heptad repeat 2 domain of gp41. mBio. 2022;13:e0358921.
14. Chen Y, Jin H, Tang X, Li L, Geng X, Zhu Y, et al. Cell membrane-anchored anti-HIV single-chain antibodies and bifunctional inhibitors targeting the gp41 fusion protein: new strategies for HIV gene therapy. Emerg Microbes Infect. 2022;11:30–49.
15. Lian X, Seiger KW, Parsons EM, Gao C, Sun W, Gladkov GT, et al. Progressive transformation of the HIV-1 reservoir cell profile over two decades of antiviral therapy. Cell Host Microbe. 2023;31:83–96.

ACKNOWLEDGEMENTS

This work was supported in part by research grants from the National Natural Science Foundation of China (No. 82230076) and the CAMS Innovation Fund for Medical Sciences (2021-I2M-1-037).

AUTHOR CONTRIBUTIONS

YG and YH drafted and edited the manuscript. YH performed the final proofreading of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Yuxian He.