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Epigenetics and Immunotherapy: New Perspective for Breaking Chronic Viral Infection

Jiabin Yan*

Center for life science, Program in cellular and molecular medicine, Boston Children's Hospital; 3 Blackfan Circle, Boston MA 0211, USA

***Corresponding author:** Jiabin Yan, Center for life science, Program in cellular and molecular medicine, Boston Children's Hospital; 3 Blackfan Circle, Boston MA 0211, USA, Tel: 617-713-8208; E-mail: bjyan2004@gmail.com; Jiabin.Yan@childrens.harvard.edu

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Abstract

Chronic or persistent viral infections, like HBV, HCV and HIV, are still a challenge for human health. More importantly, most human chronic viral infection will result in cancer, the top leading cause of death. So far antiviral drugs and immunotherapy have limited therapeutic effects on patients. New potential mechanisms of chronic viral infections are needed to explore before new effective therapeutic strategies can be put into use. Epigenetic alterations are an important hallmark of cancer. Recent studies indicate that chronic viral infections also induce epigenetic changes in the host. This review will try to summarize some of the most recent studies in these fields and explore a new strategy to break chronic viral infection by combining epigenetic therapy and immunotherapy.

Keywords: Epigenetics; Immunotherapy; Chronic viral infection; DNA methylation; Histone modification

Introduction

Epigenetics refers to heritable and potentially reversible changes in gene expression that do not change the primary sequence of genomic DNA [1]. The most extensively studied epigenetic modifications are DNA methylation and histone posttranslational modifications [2]. It is well-known that there are eight hallmarks of cancer [3]. Recently, epigenetic alterations are considered as a new hallmark of cancer [4-6]. However, more and more studies have indicated that epigenetic modifications are also associated with chronic viral infections, including HIV [7], HBV [8] and HCV [9]. In the present review, I will give a brief summary of chronic viral infection and further discuss a new strategy to break chronic viral infection by combining immunotherapy and epigenetic modifications.

Chronic Viral Infection

Most viral infections are resolved due to clearance of viruses by the host immune system. However, some viruses can establish persistent infections (in many cases initiated by an acute infection) through adoption of sophisticated relationships with their hosts and manipulation of a wide array of cellular mechanisms for their own advantage [10]. The so- called chronic viral infections are largely due to the failure of immediate viral clearance, where the host's immune system is not appropriately induced or infected viruses modify the relevant immune signaling and cellular apoptotic pathways [10].

Both DNA viruses (like HBV and HPV) and RNA viruses (like HCV and HIV) can induce chronic infections in humans [11]. Persistent viral infections are also found in nonhuman primates and mice, like simian immunodeficiency virus (SIV) and lymphocytic choriomeningitis virus (LCMV), respectively. Both SIV and LCMV infections are good models to study the mechanisms of chronic viral infection. Several mechanisms have been reported to regulate immune responses during chronic viral infection. Upregulation of inhibitory receptors on exhausted CD8 T cells is an important one. These inhibitory receptors include PD-, CTLA, LAG, etc [11].

Chronic viral infection is a challenging health problem for humans. Now it is clear that persistent viral infection will result in cancer development, like HPV and HBV [12]. It is estimated that chronic HBV infection accounts for more than 80% of HCC [13]. As for RNA viruses, HIV predisposes to the development of non-Hodgkin's lymphoma, squamous cell carcinomas, and Kaposi's sarcoma while the human T-cell lymphoma virus causes adult T-cell leukemia [14]. Viruses that induce cancer are also called tumor viruses. Tumor viruses induce malignant transformation in host through several strategies [15], including apoptosis inhibition, cell cycle deregulation, blocking of senescence, induction of genetic instability and promotion of angiogenesis.

Epigenetics in Viral Infection

Epigenetic regulation in viral infection includes two different meanings. One is that tumor viruses themselves undergo epigenetic changes; the other is that viruses cause epigenetic changes in host. Epigenetics play an important role in control of tumor viruses' life cycle and the transformation of a normal cell to a cancer cell [16]. One example is silenced HIV genomes, which is a major obstacle for a curative treatment of AIDS patients [17]. The two extensively studied epigenetics are DNA methylation and histone modification. Okamoto et al., [18] reported that HBV and HCV infection induced aberrant DNA methylation in mice with humanized livers. Lusic et al., [19] found that histone acetylation was involved in regulating of HIV-1 gene expression and factor recruitment at the LTR promoter. In addition, DNA methylation is involved in controlling viral genome of HPV [20]. Repression of tumor suppressor genes by inducing promoter hypermethylation is an important reason for carcinogenesis [,22]. More interestingly, it is found that HPV16 can epigenetically silence the interferon-κ gene [23], which indicates that chronic viral infection is able to suppress the host's innate immunity. Actually chronic viral infections also influence adaptive immune responses by epigenetic strategy. Youngblood et al., [2,25] reported that decreased

DNA methylation of the PD1 locus in $CD8^+$ T cells happened in both chronic LCMV and HIV infections. Taken together, epigenetic control of tumor viruses' life cycle is popular [26,27]. In addition, chronic viral infections are able to modify both hosts' innate and adaptive immunity by epigenetic strategy. These indicate that epigenetic therapy may be useful for interrupting and breaking chronic viral infection.

Immunotherapy for Chronic Viral Infection

Immunotherapy is a therapeutic method to boost the host's innate and adaptive immune responses to fight infectious diseases and cancer. As antiviral drugs are expensive and showing viral resistance and drug toxicity, immunotherapy is becoming a promising strategy to conquer chronic viral infection [28]. One strong evidence of HBV immunotherapy is that resolution of chronic HBV infection can be achieved by bone marrow transplantation from an immune donor [29]. Using a dually functional vector containing both an immunostimulating single-stranded RNA (ssRNA) and an HBx-silencing short hairpin RNA (shRNA), Lan et al., [30] reported that HBV-impaired hepatocyte-intrinsic innate immunity could be recovered in a mouse model. They further showed that the recovered innate immunity could overcome systemic adaptive immunotolerance in an IFN-α- and TLR7- dependent manner. Moody et al., [31] found that combination of TLR7/8 and TLR9 agonists was able to elicit the highest titers of binding, neutralizing, and antibody-dependent cellular cytotoxicity-mediating antibodies against HIV-1 envelope gp140. As the enhanced immune response was associated with the release of CXCL10 (IP-10), it indicated that the TLR adjuvant formulation may have optimally stimulated innate and adaptive immunity to elicit high titers of antibodies.

There are different types of immunotherapy for chronic viral infection, including cytokine delivery [32], therapeutic vaccines and blocking antibodies targeting co-inhibitory receptors such as PD-1 [33]. Therapeutic vaccines are one major immunotherapy strategy, where a variety of platforms and approaches including DNA, viral vectors, dendritic cells and peptides are tested, together with some adjuvants and immune modulators [34]. It is well-accepted that combination therapy will have better effect. One example is that combining antagonists of IL-10R or PD-1 with a therapeutic vaccine strengthened the effects in a murine model of persistent lymphocytic choriomeningitis virus (LCMV) infection [35]. Table 1 lists some of the representative studies for immunotherapy on chronic virus infections.

Virus	Reagent	References
HIV	3BNC117	Caskey et al. [36]
HBV	IFN-α	Lucifora et al. [37]
HCV	Adenoviral vaccine	Barnes et al. [38]
LCMV	PD-L1 blockade	Penaloza-MacMaster et al. [35]

Table 1: Immunotherapy for chronic viral infections.

Epigenetic Therapy for Chronic Viral Infection

During epigenetic alteration, two most studied enzymes are DNA methyl transferase (DNMT) and histone deacetylase (HDAC). Accordingly, the best characterized epigenetic drugs are DNMT inhibitors and HDAC inhibitors. To date, three DNMT inhibitors are FDA-approved. They are 5-azacitidine, decitabine and Farydak (panobinostat). As for HDAC inhibitors, Vorinostat (SAHA, Zolina), romidepsin (Istodax, FK228, FR901228, depsipeptide) and belinostat (Beleodaq, PXD-101) have been approved for cancer therapy by FDA [39].

So far most epigenetic therapies are studied for cancer. Besides the modifying effect on gene expression, recent studies also show that epigenetic drugs are good immunomodulators. They influence both innate and adaptive immune responses [4]. Many reports have shown that epigenetic drugs are able to improve the costimulatory properties of cancer cells by up regulating surface expression of CD40, CD80, CD86, and ICAM1 and restore their sensitivity to immune cell triggered apoptosis by enhancing the expression of death-inducing receptors [40-42] . Zhang et al., [43] reported that HDAC inhibitors restored function of defective $CD8^+$ T cells from chronic LCMV infection. Archin et al., [44] reported that selective HDAC inhibitors could activate latency HIV infection, which suggests a clinical strategy to target persistent HIV infection. The same group [45] further showed that administration of vorinostat disrupted HIV-1 latency in patients on antiretroviral therapy. This study is of great significance as it indicates that eradication of latent HIV infection is possible. Wei et al. [46] reported that romidepsin could induce HIV expression in CD4 T cells from HIV patients. Recently Tian et al., [47] showed that Telbivudine treatment corrected HBV- induced epigenetic alterations in liver cells of patients with chronic hepatitis B. HBV nuclear cccDNA accumulates in hepatocyte nuclei as a stable minichromosome organized by histone and non-histone viral and cellular proteins [48]. Identification of the molecular mechanisms regulating cccDNA stability and its transcriptional activity at the RNA, DNA and epigenetic levels may reveal new potential therapeutic targets. Taken together, considering the epigenetic regulation of latent HIV and HBV cccDNA, epigenetic therapy may be a promising strategy to conquer chronic/persistent viral infection. Table 2 lists some of the representative studies for epigenetic therapy on chronic viral infections.

Virus	Reagent	References
HIV	Vorinostat	Archin et al. [45]

	Panobinostat Decitabine	Rasmussen et al. [49] Fernandez and Zeichner [50]
HBV	p300/CBP inhibitor	Tropberger et al. [8]
HCV	Decitabine	Quan et al. [51]
LCMV	Valproic acid	Zhang et al. [43]

Table 2: Epigenetic therapy for chronic viral infections.

Breaking Chronic Viral Infection by Combining Epigenetic Drugs and Immunotherapy

Many studies have shown that either antiviral drugs or immunotherapy alone have limited effects on patients with chronic viral infection. Kelly et al., [52] reported that immunotherapy with therapeutic vaccine alone failed to restore T cell immunity in HCV infected patients. Kang et al., [53] showed that combination of EGCG and DNA vaccination led to an enhanced tumor-specific T-cell immune response and enhanced antitumor effects, resulting in a higher cure rate than either immunotherapy or EGCG alone. These studies suggest that combining epigenetic therapy and immunotherapy have better therapeutic effects on tumor. It is promising to try the same strategy on patients with chronic viral infection. Tropberger et al., [8] found that HBV cccDNA chromatin was modulated by innate immunity and manipulated with an epigenetic agent. Their findings provide rational evidence for treating chronic HBV patients by combining immunotherapy and epigenetic therapy.

It should be noted that, epigenetic reagents may have more effects on viral control besides modulating gene expression. One recent paper [54] showed that an epigenetic chemical epigallocatechin-3-gallate (EGCG), the main polyphenol compound of green tea, was able to inhibit HCV entry.

One specific goal of epigenetic therapy is to restore normal DNA methylation patterns and to prevent the cells from acquiring further methylation in DNA that could lead to silencing of genes crucial for normal cell function. Concerns appear immediately about epigenetic therapy with DNMT inhibitors and HDAC inhibitors: Is that effect malignance-directed *in vivo* or universal? In other words, the use of demethylating agents may aggravate the situation by further decreasing the level of methylation and activation of genes that are potentially deleterious, like oncogenes. One recent report [55] showed that 5-Aza-CdR was able to modulate immune- related genes in mouse tumor while normal tissues were substantially unaffected. Cheng et al [50] reported that zebularine could be selective toward cancer cells rather than normal cells [56]. Although safety concerns still exist [57], epigenetic therapy combined with current immunotherapy methods (including some antiviral drugs) may be promising to break chronic viral infection and eradicate viruses from host completely.

Conclusion

Epigenetics provide new strategy to study chronic viral infection, especially those caused by tumor viruses. It is necessary to explore as many as possible molecular mechanisms of epigenetic drugs on cancer and virus infection treatment. As most current epigenetic therapies are carried out on cancer patients, it is interesting to check whether the similar recipe will work for patients with chronic viral infection. Finally and most importantly, combining epigenetic therapy together with immunotherapy may provide a promising strategy to break chronic viral infection and induce appropriate immune responses to eradicate viruses from the host.

Conflicts of Interest

The author declares no conflict of interest.

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References

1. Sigalotti L, Covre A, Fratta E, Parisi G, Colizzi F, et al. (2010) Epigenetics of human cutaneous melanoma: setting the stage for new therapeutic strategies. J Transl Med 8: 56.
2. Maio M, Covre A, Fratta E, Di Giacomo AM, Taverna P, et al. (2015) Molecular Pathways: At the Crossroads of Cancer Epigenetics and Immunotherapy. Clin Cancer Res 21: 4040-4047.
3. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-674.
4. Sigalotti L, Fratta E, Coral S, Maio M (2014) Epigenetic drugs as immunomodulators for combination therapies in solid tumors. Pharmacol Ther 142: 339-350.
5. Mair B, Kubicek S, Nijman SM (2014) Exploiting epigenetic vulnerabilities for cancer therapeutics. Trends Pharmacol Sci 35: 136-145.
6. Sarkar S, Horn G, Moulton K, Oza A, Byler S, et al. (2013) Cancer development, progression, and therapy: an epigenetic overview. Int J Mol Sci 14: 21087-21113.
7. Kumar A, Darcis G, Van Lint C, Herbein G (2015) Epigenetic control of HIV-1 post integration latency: implications for therapy. Clin Epigenetics 7: 103.
8. Tropberger P, Mercier A, Robinson M, Zhong W, Ganem DE, et al. (2015) Mapping of histone modifications in episomal HBV cccDNA uncovers an unusual chromatin organization amenable to epigenetic manipulation. Proc Natl Acad Sci U S A 112: E5715-5724.
9. El-Ekiaby NM, Mekky RY, El Sobky SA, Elemam NM, El-Sayed M, et al. (2015) Epigenetic harnessing of HCV via modulating the lipid droplet-protein, TIP47, in HCV cell models. FEBS Lett 589: 2266-2273.
10. Kane M, Golovkina T (2010) Common threads in persistent viral infections. J Virol 84: 4116-4123.
11. Virgin HW, Wherry EJ, Ahmed R (2009) Redefining chronic viral infection. Cell 138: 30-50.
12. Moore PS, Chang Y (2010) Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer 10: 878-889.
13. Di Bisceglie AM (2009) Hepatitis B and hepatocellular carcinoma. Hepatology 49: S56-60.

14. Butel JS (2000) Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis 21: 405-426.
15. Hoppe-Seyler F, Hoppe-Seyler K (2011) Emerging topics in human tumor virology. Int J Cancer 129: 1289-1299.
16. Milavetz BI, Balakrishnan L (2015) Viral epigenetics. Methods Mol Biol 1238: 569-596.
17. Ay E, Banati F, Mezei M, Bakos A, Niller HH, et al. (2013) Epigenetics of HIV infection: promising research areas and implications for therapy. AIDS Rev 15: 181-188.
18. Okamoto Y, Shinjo K, Shimizu Y, Sano T, Yamao K, et al. (2014) Hepatitis virus infection affects DNA methylation in mice with humanized livers. Gastroenterology 146: 562-572.
19. Lusic M, Marcello A, Cereseto A, Giacca M (2003) Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter. EMBO J 22: 6550-6561.
20. Johannsen E, Lambert PF (2013) Epigenetics of human papillomaviruses. Virology 445: 205-212.
21. Hino R, Uozaki H, Murakami N, Ushiku T, Shinozaki A, et al. (2009) Activation of DNA methyltransferase 1 by EBV latent membrane protein 2A leads to promoter hypermethylation of PTEN gene in gastric carcinoma. Cancer Res 69: 2766-2774.
22. Laurson J, Khan S, Chung R, Cross K, Raj K (2010) Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein. Carcinogenesis 31: 918-926.
23. Rincon-Orozco B, Halec G, Rosenberger S, Muschik D, Nindl I, et al. (2009) Epigenetic silencing of interferon-kappa in human papillomavirus type 16-positive cells. Cancer Res 69: 8718-8725.
24. Youngblood B, Noto A, Porichis F, Akondy RS, Ndhlovu ZM, et al. (2013) Cutting edge: Prolonged exposure to HIV reinforces a poised epigenetic program for PD-1 expression in virus-specific CD8 T cells. J Immunol 191: 540-544.
25. Youngblood B, Oestreich KJ, Ha SJ, Duraiswamy J, Akondy RS, et al. (2011) Chronic virus infectionenforces demethylation of the locus that encodes PD-1 in antigen-specific CD8(+) T cells. Immunity 35: 400-412.
26. Zhang X, Hou J, Lu M (2013) Regulation of hepatitis B virus replication by epigenetic mechanisms and microRNAs. Front Genet 4: 202.
27. Knipe DM, Lieberman PM, Jung JU, McBride AA, Morris KV, et al. (2013) Snapshots: chromatin control of viral infection. Virology 435: 141-156.
28. Grimm D, Heeg M, Thimme R (2013) Hepatitis B virus: from immunobiology to immunotherapy. Clin Sci (Lond) 124: 77-85.
29. Ilan Y, Nagler A, Adler R, Tur-Kaspa R, Slavin S, et al. (1993) Ablation of persistent hepatitis B by bone marrow transplantation from a hepatitis B-immune donor. Gastroenterology 104: 1818-1821.
30. Lan P, Zhang C, Han Q, Zhang J, Tian Z (2013) Therapeutic recovery of hepatitis B virus (HBV)-induced hepatocyte-intrinsic immune defect reverses systemic adaptive immune tolerance. Hepatology 58: 73-85.
31. Moody MA, Santra S, Vandergrift NA, Sutherland LL, Gurley TC, et al. (2014) Toll-like receptor 7/8 (TLR7/8) and TLR9 agonists cooperate to enhance HIV-1 envelope antibody responses in rhesus macaques. J Virol 88: 3329-3339.
32. Nanjappa SG, Kim EH, Suresh M (2011) Immunotherapeutic effects of IL-7 during a chronic viral infection in mice. Blood 117: 5123-5132.
33. Salem ML, El-Badawy A (2015) Programmed death-1/programmed death-L1 signaling pathway and its blockade in hepatitis C virus immunotherapy. World J Hepatol 7: 2449-2458.
34. Fisher AK, Voronin Y, Jefferys R (2014) Therapeutic HIV vaccines: prior setbacks, current advances, and future prospects. Vaccine 32: 5540-5545.
35. Penaloza-MacMaster P, Kamphorst AO, Wieland A, Araki K, Iyer SS, et al. (2014) Interplay between regulatory T cells and PD-1 in modulating T cell exhaustion and viral control during chronic LCMV infection. J Exp Med 211: 1905-1918.
36. Caskey M, Klein F, Lorenzi JC, Seaman MS, West AP Jr, et al. (2015) Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature 522: 487-491.
37. Lucifora J, Xia Y, Reisinger F, Zhang K, Stadler D, et al. (2014) Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 343: 1221-1228.
38. Barnes E, Folgori A, Capone S, Swadling L, Aston S, et al. (2012) Novel adenovirus-based vaccines induce broad and sustained T cell responses to HCV in man. Sci Transl Med 4: 115ra1.
39. Mottamal M, Zheng S, Huang TL, (2015) Histone deacetylase inhibitors in clinical studies as templates for new anticancer agents. Molecules 20: 3898-3941.
40. Srivastava P, Paluch BE, Matsuzaki J, James SR, Collamat-Lai G, et al. (2014) Immunomodulatory action of SGI-110, a hypomethylating agent, in acute myeloid leukemia cells and xenografts. Leuk Res 38: 1332-1341.
41. Coral S, Parisi G, Nicolay HJ, Colizzi F, Danielli R, et al. (2013) Immunomodulatory activity of SGI-110, a 5-aza-2'-deoxycytidine-containing demethylating dinucleotide. Cancer Immunol Immunother 62: 605-614.
42. Murakami T, Sato A, Chun NA, Hara M, Naito Y, et al. (2008) Transcriptional modulation using HDACi depsipeptide promotes immune cell-mediated tumor destruction of murine B16 melanoma. J Invest Dermatol 128: 1506-1516.
43. Zhang F, Zhou X, DiSpirito JR, Wang C, Wang Y, et al. (2014) Epigenetic manipulation restores functions of defective CD8+ T cells from chronic viral infection. Mol Ther 22: 1698-1706.
44. Archin NM, Keedy KS, Espeseth A, Dang H, Hazuda DJ, et al. (2009) Expression of latent human immunodeficiency type 1 is induced by novel and selective histone deacetylase inhibitors. AIDS 23: 1799-1806.
45. Archin NM, Liberty AL, Kashuba AD, Choudhary SK, Kuruc JD, et al. (2012) Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. Nature 487: 482-485.
46. Wei DG, Chiang V, Fyne E, Balakrishnan M, Barnes T, et al. (2014) Histone deacetylase inhibitor romidepsin induces HIV expression in CD4 T cells from patients on suppressive antiretroviral therapy at concentrations achieved by clinical dosing. PLoS Pathog 10: e1004071.
47. Tian Y, Ni D, Yang W, Zhang Y, Zhao K, et al. (2014) Telbivudine treatment corrects HBV-induced epigenetic alterations in liver cells of patients with chronic hepatitis B. Carcinogenesis 35: 53-61.
48. Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, et al. (2009) Control of cccDNA function in hepatitis B virus infection. J Hepatol 51: 581-592.
49. Rasmussen TA, Tolstrup M, Brinkmann CR, Olesen R, Erikstrup C, et al. (2014) Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/, single group, clinical trial. Lancet HIV 1: e13-21.
50. Fernandez G, Zeichner SL (2010) Cell line-dependent variability in HIV activation employing DNMT inhibitors. Virol J 7: 266.
51. Quan H, Zhou F, Nie D, Chen Q, Cai X, et al. (2014) Hepatitis C virus core protein epigenetically silences SFRP1 and enhances HCC aggressiveness by inducing epithelial-mesenchymal transition. Oncogene 33: 2826-2835.
52. Kelly C, Swadling L, Capone S, Brown A, et al. (2015) Chronic Hepatitis C Virus infection subverts vaccine induced T-cell immunity in humans. Hepatology .
53. Kang TH, Lee JH, Song CK, Han HD, Shin BC, et al. (2007) Epigallocatechin-3-gallate enhances CD8+ T cell-mediated antitumor immunity induced by DNA vaccination. Cancer Res 67: 802-811.
54. Ciesek S, von Hahn T, Colpitts CC, Schang LM, Friesland M, et al. (2011) The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry. Hepatology 54: 1947-1955.
55. Coral S, Covre A, Nicolay HJ, Parisi G, Rizzo A, et al. (2012) Epigenetic remodelling of gene expression profiles of neoplastic and normal tissues: immunotherapeutic implications. Br J Cancer 107: 1116-1124.
56. Cheng JC, Yoo CB, Weisenberger DJ, Chuang J, Wozniak C, et al. (2004) Preferential response of cancer cells to zebularine. Cancer Cell 6: 151-158.
57. Gius D, Cui H, Bradbury CM, Cook J, Smart DK, et al. (2004) Distinct effects on gene expression of chemical and genetic manipulation of the

cancer epigenome revealed by a multimodality approach. Cancer Cell 6: 361-371.