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CONTRACTING ORGANIZATION: Louisiana State University Health Sciences Center, New Orleans, LA 70112-7021

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14. ABSTRACT

During the whole funding period (2015-2018), we have completed all the 3 Specific Aims as listed in the SOW forms above. Our results have illuminated the complicated mechanisms through which the HGF/c-MET pathway regulates KSHV+ PEL cell survival. Our exciting in vivo data have provided the framework for development and implementation of clinical trials for evaluating strategies targeting HGF/c-MET (alone or combination of other therapies) for the treatment of lymphoma in HIV-infected patients including military personnel. Until January 2019, we have totally published 25 peer-reviewed articles about the molecular mechanisms of KSHV viral oncogenesis, and developing novel therapeutic strategies against these malignancies. In most of these publications, I am served as the corresp onding or co-corresponding author. We also have published 9 meeting abstracts on national or international meetings . With the support by this DOD award, I have obtained several intramural and extramural funding, including a NIH/NCI RO1 funding (as PI) and a NIH COBRE subproject (as the project leader).

15. SUBJECT TERMS
HGF, c-MET, KSHV, primary effusion lymphoma, apoptosis, cell cycle

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1. INTRODUCTION

Viruses are the most common cause of lymphoma in patients with immune dysfunction, and virus-associated lymphomas incur high mortality for these patients due to a lack of effective therapeutic strategies. Studies in this proposal are designed to elucidate mechanisms for regulation of AIDS/KSHV-associated lymphoma (in particular KSHV+ PEL) pathogenesis by the oncogenic protein HGF/c-MET, and whether targeting HGF/c-MET reduce virus-associated lymphoma progression *in vivo*.

2. KEYWORDS

HGF, c-MET, KSHV, primary effusion lymphoma, apoptosis, cell cycle

3. ACCOMPLISHMENTS

3.1. What were the major goals of the project?

There are 3 specific aims in this project, Aim 1: To identify the complex mechanisms of the HGF/c-MET pathway controlling cell survival/growth for PEL tumor cells. Aim 2: To understand the mechanisms viral oncogenic proteins used to activate the HGF/c-MET pathway. Aim 3: To determine whether a selective small-molecule inhibitor of c-MET, PF-2341066, can repress PEL progression and/or reduce established tumor in an immune-deficient xenograft mice model.

The following are the condition of subtask completion as indicated in SOW:

Specific Aim 1(specified in proposal)	**Timeline**	**Site 1**
Major Task 1	Months	
Subtask 1: *HGF/c-MET affects viral gene expression (completed)*	2-3	Dr. Qin
Subtask 2: *HGF/c-MET affects downstream signaling pathways (completed)*	3-4	Dr. Qin
Subtask 3: *HGF/c-MET affects cell cycle checkpoints (completed)*	2-3	Dr. Qin
Subtask 4: *HGF/c-MET affects HGF secretion from PEL cells (completed)*	1-2	Dr. Qin
Specific Aim 2 (specified in proposal)	**Timeline**	**Site 1**
Major Task 2	Months	
Subtask 1: *viral proteins are essential for activation of HGF/c-MET (completed)*	2-3	Dr. Qin
Subtask 2: *key domain or amino acid residues essential for activation of HGF/c-MET (completed)*	4-6	Dr. Qin

Subtask 3: *the role of c-MET phosphorylation in signaling activation (completed)*	2-3	Dr. Qin
Specific Aim 3 (specified in proposal)	**Timeline**	**Site 1**
Major Task 3	Months	
Subtask 1: *c-MET inhibitor prevents PEL development in NOD/SCID mice model (completed)*	4-5	Dr. Qin
Subtask 2: *c-MET inhibitor reduces established PEL progression in NOD/SCID mice model (completed)*	5-7	Dr. Qin

3.2. What was accomplished under these goals?

During the whole funding period (2015-2018), we have completed all the 3 Specific Aims as listed in the SOW forms above. Until January 2019, we have totally published 25 peer-reviewed articles about the molecular mechanisms of KSHV viral oncogenesis, and developing novel therapeutic strategies against these malignancies. In most of these publications, I am served as the corresponding or co-corresponding author. We also have published 9 meeting abstracts on national or international meetings (please see below details in **PRODUCTS**). Our major findings include:

1) Targeting HGF/c-MET pathway induced KSHV+ PEL cell apoptosis through cell cycle arrest and DNA damage.
2) Identifying downstream genes controlled by HGF/c-MET pathway in KSHV+ PEL cell-lines. One of genes, ribonucleotide reductase, represents a novel therapeutic target in PEL.
3) Selective c-MET inhibitor treatment effectively preventing PEL expansion in the xenograft model. Targeting HGF/c-MET pathway may also have promising therapeutic effects on Kaposi's Sarcoma, another cancer caused by KSHV.
4) Plexin-B1 is required for c-MET phosphorylation and related cellular functions within PEL cells, while our results indicate that mutations are not the major mechanism responsible for c-MET activation in PEL.

3.3. What opportunities for training and professional development has the project provided?

I have trained 3 postdoctors in my lab: Dr. Lu Dai has published 22 papers during this funding period (as the first author in most publications); Dr. Bao Quoc Lam has published 2 papers; and Dr. Jungang Chen has published 2 papers. We also have displayed our data in many national or international meetings such as International Conference on EBV & KSHV. With the support by this DOD award, I have obtained several intramural and extramural funding, including a LA CaTS Pilot Funding, a LSU LIFT funding, a Leukemia Research Foundation pilot funding as well as a NIH/NCI RO1 funding (all as PI) and a NIH COBRE subproject (as project leader), the details have been listed below.

3.4. How were the results disseminated to communities of interest?

Nothing to Report.

3.5. What do you plan to do during the next reporting period to accomplish the goals?

Since all the aims have been completed, we ask this project be closed although we have asked 1-y no cost of extension previously.

4. IMPACT

4.1. What was the impact on the development of the principal discipline(s) of the project?

Our results have illuminated the complicated mechanisms through which the HGF/c-MET pathway regulates KSHV+ PEL cell survival. Our exciting *in vivo* data have provided the framework for development and implementation of clinical trials for evaluating strategies targeting HGF/c-MET (alone or combination of other therapies) for the treatment of lymphoma in HIV-infected patients including military personnel.

4.2. What was the impact on other disciplines?

Nothing to Report.

4.3. What was the impact on technology transfer?

Nothing to Report.

4.4. What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

Dr. Zhiqiang Qin, the PI of this project has recently moved to UAMS, Little Rock, AR from LSUHSC, New Orleans, LA.
Dr. Chris Parsons, the mentor of this project has left LSUHSC-NO and is no longer served as key personnel in this project.

6. PRODUCTS

6.1. Journal publications (total 25, # as the corresponding author, all have acknowledgement of DoD federal support):

1. Dai L., Trillo-Tinoco J., Cao Y., Bonstaff K., Doyle L., Valle LD., Whitby D., Parsons C., Reiss K., Zabaleta J., **Qin, Z #**. Targeting HGF/c-MET induces cell cycle arrest, DNA damage and apoptosis for primary effusion lymphoma. *Blood,* 2015;126(26):2821-31. PMCID: PMC4692142.
2. Dai L., Trillo-Tinoco J., Bai A., Chen Y., Bielawski J., Valle LD., Smith CD., Ochoa AC., **Qin, Z #**., Parsons C #**.** Ceramides promote apoptosis for virus-infected lymphoma cells through induction of ceramide synthases and viral lytic gene expression. *Oncotarget*. 2015;6(27):24246-60. PMCID: PMC4695183.
3. Dai, L., Chen Y., Toole BP., Parsons, C., **Qin, Z #**. Induction of Hyaluronan Production by oncogenic KSHV and the Contribution to Viral Pathogenesis in AIDS Patients. *Cancer Lett*. 2015;362(2):158-66. PMCID: PMC4410079.
4. Dai, L., Cao Y., Chen Y., Kaleeba, J.A.R., Zabaleta**,** J., **Qin, Z #**. Genomic analysis of xCT-mediated regulatory network: identification of novel targets against AIDS-associated lymphoma. *Oncotarget*. 2015;6(14):12710-22. PMCID: PMC4494968.Dai L, Bai L, Lin Z, Yang L, Flemington EK, Zabaleta J, **Qin Z** #. Transcriptomic analysis of KSHV-infected primary oral fibroblasts: the role of interferon-induced genes in the latency of oncogenic virus. *Oncotarget,* 2016 May 30. doi: 10.18632/oncotarget.9720. [Epub ahead of print]. PMID: 27363016.

5. Dai L, Qiao J, Struckhoff AP, Nguyen D, Del Valle L, Parsons C, Ochoa AC, Toole BP, Renne R, **Qin Z** #. Role of heme oxygenase-1 in the pathogenesis and tumorigenicity of Kaposi's sarcoma-associated herpesvirus. *Oncotarget*. 2016;7(9):10459-71. PMCID: PMC4891132.

6. **Qin Z #,** Cao Y, Dai L #. Genomic analysis of xCT-regulatory network in KSHV+ primary effusion lymphomas. *Genomics Data*. 2016; 8: 16–7. PMCID: PMC4818344.

7. Dai L., Trillo-Tinoco J., Chen Y., Bonstaff K., Valle LD., Parsons C., Ochoa AC., Zabaleta J., Toole BP., **Qin, Z #**. CD147 and downstream ADAMTSs promote the tumorigenicity of Kaposi sarcoma-associated herpesvirus. *Oncotarget*, 2016;7(4):3806-18. PMCID: PMC4826171.

8. Lam BQ, Dai L, **Qin Z** #. The role of HGF/c-MET signaling pathway in lymphoma. *J Hematol Oncol*. 2016;9(1):135. PMCID: PMC5141645

9. Dai L, Lin Z, Cao Y, Chen Y, Xu Z, **Qin Z #.** Targeting EIF4F complex in non–small cell lung cancer cells. *Oncotarget*. 2017 Jun 8. doi: 10.18632/oncotarget.18413. [Epub ahead of print]. PMID: 28607208.

10. Dai L, Lin Z, Qiao J, Zabaleta J, Flemington EK, **Qin Z #.** Ribonucleotide reductase represents a novel therapeutic target in primary effusion lymphoma. *Oncogene*. 2017 May 1. doi: 10.1038/onc.2017.122. [Epub ahead of print]. PMID: 28459467.

11. Dai L, Cao Y, Wei Jiang, Zabaleta J, Jiang W, Zhongmin Liu, Qiao J #, **Qin Z #**. KSHV co-infection down-regulates HPV16 E6 and E7 from cervical cancer cells. *Oncotarget*. 2017;8(22):35792-35803. PMCID: PMC5482618

12. Lam BQ, Dai L, Li L, Qiao J, Lin Z #, **Qin Z** #. Molecular mechanisms of activating c-MET in KSHV+ primary effusion lymphoma. *Oncotarget*. 2017; 8(11):18373-18380. PMCID: PMC5392335

13. Cao Y, Qiao J, Lin Z, Zabaleta J, Dai L, **Qin Z** #. Up-regulation of tumor suppressor genes by exogenous dhC16-Cer contributes to its anti-cancer activity in primary effusion lymphoma. *Oncotarget*. 2017; 8(9):15220-15229. PMCID: PMC5362481

14. Bonsignore L, Passelli K, Pelzer C, Perroud M, Konrad A, Thurau M, Stürzl M, Dai L, Trillo-Tinoco J, Del Valle L, **Qin Z** #, Thome M #. A role for MALT1 activity in Kaposi's sarcoma-associated herpes virus latency and growth of primary effusion lymphoma. *Leukemia*, 2017; 31(3):614-624. PMCID: PMC5339436

15. Dai L, Bai A, Smith CD, Rodriguez PC, Yu F #, **Qin Z** #. ABC294640, a novel sphingosine kinase 2 inhibitor induces oncogenic virus infected cell autophagic death and represses tumor growth. *Mol Cancer Ther*. 2017;16(12):2724-2734.

16. Luo Z, Zhou Z, Ogunrinde E, Zhang T, Li Z, Martin L, Wan Z, Wu H, **Qin Z,** Ou T, Zhang J, Ma L, Liao G, Heath S, Huang L, Jiang W *. The effect of plasma auto-IgGs on CD4+ T cell apoptosis in viral-suppressed HIV-infected patients under antiretroviral therapy. *J Leukoc Biol*. 2017;102(6):1481-1486.

17. Dai L, Lin Z, Jiang W, Flemington EK, **Qin Z #.** Lipids, lipid metabolism and Kaposi's sarcoma-associated herpesvirus pathogenesis. *Virol Sin.* 2017;32(5):369-375.

18. Qiao J, Cao Y, Zabaleta J, Yang L, Dai L #, **Qin Z** #. Regulation of virus-associated lymphoma growth and gene expression by bacterial quorum sensing molecules. *J Virol*. 2018 May 9. pii: JVI.00478-18. doi: 10.1128/JVI.00478-18. [Epub ahead of print].

19. Dai L, Chen J, Cao Y, Del Valle L, **Qin Z** #. Ribonucleotide reductase inhibitor 3-AP induces oncogenic virus–infected cell death and represses tumor growth. *J Cancer*. 2018, 9(23):4503-4509.

20. Xu W, Luo Z, Alekseyenko AV, Martin L, Wan Z, Ling B, **Qin Z,** Heath SL, Maas K, Cong X, Jiang W. Distinct systemic microbiome and microbial translocation are associated with plasma level of anti-CD4 autoantibody in HIV infection. *Sci Rep.* 2018 Aug 27;8(1):12863. doi: 10.1038/s41598-018-31116-y.

21. Dai L, Del Valle L, Miley W, Whitby D, Ochoa AC, Flemington EK, **Qin Z** #. Transactivation of human endogenous retrovirus K (HERV-K) by KSHV promotes Kaposi's Sarcoma development. *Oncogene*. 2018 May 10. doi: 10.1038/s41388-018-0282-4. [Epub ahead of print].

22. Dai L, Qiao J, Del Valle L, **Qin Z** #. KSHV co-infection regulates HPV16+ cervical cancer cells pathogenesis *in vitro* and *in vivo*. *Am J Cancer Res*. 2018;8(4):708-714.

23. Dai L, Smith CD, Foroozesh M, Miele L, **Qin Z** #. The sphingosine kinase 2 inhibitor ABC294640 displays anti-non-small cell lung cancer activities *in vitro* and *in vivo*. *Int J Cancer*. 2018;142(10):2153-2162.

24. Dai L, Zhao M, Jiang W, Lin Z, Del Valle L, **Qin Z** #. KSHV co-infection, a new co-factor for HPV-related cervical carcinogenesis? *Am J Cancer Res*. 2018;8(11):2176-2184.

25. Chen J, Foroozesh M, **Qin Z** #. Transactivation of Human Endogenous Retroviruses by Tumor Viruses and Their Functions in Virus-associated Malignancies. *Oncogenesis*, 2019;8(1):6. doi: 10.1038/s41389-018-0114-y.

6.2. Other publications, conference papers, and presentations:

1. Dai L, Trillo-Tinoco J, Del Vella L, Zabaleta J and **Qin Z #.** "Targeting HGF/c-MET pathway in KSHV+ Primary Effusion Lymphoma". (Oral presentation) 18th International Workshop on Kaposi's Sarcoma Associated Herpesvirus (KSHV) and Related Agents, Miami, FL, US, 2015.

2. **Qin Z,** Dai L, Trillo-Tinoco J, Del Vella L, Smith CD and Parsons C #. "Targeting Sphingolipid Metabolism in Virus-associated Lymphoma". (Oral presentation) 18th International Workshop on Kaposi's Sarcoma Associated Herpesvirus (KSHV) and Related Agents, Miami, FL, US, 2015.

3. Dai L, Trillo-Tinoco J, Lin Z, Del Valle L, Flemington EK and **Qin Z** #. "Role of HERV-K Transactivation in KSHV-related Malignancies". (Oral presentation) 19th International Workshop on Kaposi's Sarcoma Associated Herpesvirus (KSHV) and Related Agents, Los Angeles, CA, US, 2016.

4. Bonsignore L, Passelli K, Pelzer C, Konrad A, Thurau M, Stürzl M, Dai L, Trillo-Tinoco J, Del Valle L, **Qin Z** # and Thome M #. "A Role for MALT1 Activity in Kaposi's Sarcoma-Associated Herpesvirus Latency and Growth of Primary Effusion Lymphoma". The 2016 ASH Meeting on Lymphoma Biology in Colorado Springs, CO, US, 2016.

5. **Qin Z #,** Dai L and Toole BP. "The Role of Hyaluronan and Signaling in Virus-associated Lymphoma Chemoresistance". The 2016 ASH Meeting on Lymphoma Biology in Colorado Springs, CO, US, 2016.

6. Dai L, Trillo-Tinoco J, Lin Z, Del Valle L, Flemington EK, **Qin Z** #. "Role of HERV-K Transactivation in KSHV-related Malignancies". 28th International Workshop on Retroviral Pathogenesis, New Orleans, LA, US. 2016

7. Dai L, Foroozesh M, **Qin Z #**. "Developing new ceramide analogs against AIDS-related lymphoma". AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. Philadelphia, PA, US, 2017.

8. Dai L, Goldstein A and **Qin Z #.** "Periodontal Bacteria Promote the Pathogenesis of Kaposi's Sarcoma-associated Herpesvirus in HIV+ Patients". American Society for Microbiology South Central Branch Annual Meeting, Little Rock, AR, US, 2017.

9. Dai L, Del Valle L, Yang L and **Qin Z #**. "Pathogenic Bacteria PAMPs Promote Oncogenic Virus Pathogenesis". International Conference on EBV & KSHV, Madison, WI, US, 2018.

6.3. Other Products:

We have deposited our microarray data: 1) the regulatory network of HGF/c-MET pathway in KSHV+ PEL cell-lines to Gene Expression Omnibus (GEO) database (Accession number: GSE70594); 2) the regulatory network of RRM2 inhibitor 3-AP treated KSHV+ PEL cell-lines to GEO database (Accession number: GSE91389).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.1. What individuals have worked on the project?

Name:	*Zhiqiang Qin*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Qin is responsible for experimental design, data analysis, animal work and manuscript preparation*
Funding Support:	*NIH/NCI RO1 (NO-overlapping with the current project)*

Name:	*Lu Dai*
Project Role:	*Senior postdoctoral researcher*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Dai is responsible for cell culture, qRT-PCR, immunoblots, animal work etc*
Funding Support:	*N/A*

7.2. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

For Dr. Zhiqiang Qin:

Completed:

P20GM103501 (PI: Augusto Ochoa) 01/01/2012-06/30/2015 2.5 CM

NIH-NIGMS
Mentoring Translational Researchers in Louisiana
Project 12: KSHV regulation of emmprin, drug resistance, and tumor progression
Role: Pilot Project Leader

Leukemia Research Foundation 07/01/2016-06/30/2017 1.0CM

Targeting sphingolipid metabolism in AIDS-related lymphomas Role: Principal Investigator

LA CaTS Pilot Funding 02/01/2017-01/31/2018 0.8 CM

Developing new ceramide analogs as therapeutic agents against AIDS-related lymphomas
Role: Principal Investigator

NIH/NIGMS COBRE 01/01/2017-12/15/2018 2.4 CM
P20GM121288 (PI: Krzysztof Reiss)
Center for Translational Viral Oncology
Tier 1 Project 1 title: Role of HERV-K reactivation in AIDS-related Kaposi's Sarcoma
Role: Tier 1 project 1 leader
(I have graduated from this COBRE since I have obtained my own RO1 funding)

LSU LIFT funding 07/01/2017-12/31/2018 (6-month no cost extension) 0.8 CM

Developing new ceramide analogous "lead-compounds" against AIDS-related lymphomas in vivo
Role: Principal Investigator

Active:

NIH/NCI RO1 05/01/2018-04/30/2023 3.6 CM
1R01CA228166-01
Title: Periodontal bacteria enhance oral KSHV pathogenesis and Kaposi's Sarcoma development in HIV+ patients
Role: Principal Investigator
NO-overlapping with the current project

7.3. What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

N/A

9. APPENDICES

The original copies of 2 new journal articles recently published are attached, while the other 23 published papers have already been attached in 2016, 2017 and 2018 Annual report, respectively.

Am J Cancer Res 2018;8(11):2176-2184
www.ajcr.us /ISSN:2156-6976/ajcr0086855

Review Article

KSHV co-infection, a new co-factor for HPV-related cervical carcinogenesis?

Lu Dai[1,5], Mengmeng Zhao[3], Wei Jiang[4], Zhen Lin[3], Luis Del Valle[2], Zhiqiang Qin[1,5]

Departments of [1]Genetics, [2]Pathology, Louisiana State University Health Sciences Center, Louisiana Cancer Research Center, 1700 Tulane Ave, New Orleans, LA 70112, USA; [3]Department of Pathology, Tulane University Health Sciences Center, Tulane Cancer Center, 1700 Tulane Ave, New Orleans, LA 70112, USA; [4]Department of Microbiology and Immunology, Division of Infectious Diseases, Department of Medicine, Medical University of South Carolina, 173 Ashley Ave., Charleston, SC 29425, USA; [5]Department of Pediatrics, Research Center for Translational Medicine and Key Laboratory of Arrhythmias, East Hospital, School of Medicine, Tongji University, Shanghai 200120, China

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Abstract: High-risk human papillomavirus (HPV) infection is the etiological agent of cervical cancer and some other cancers. Kaposi sarcoma-associated herpesvirus (KSHV) represents a principal causative agent of several human cancers arising in those immunocompromised patients. In fact, KSHV DNA has been detected in the female genital tract, and this virus may share some transmission routes with HPV, although the detection rate of KSHV in cervical samples is very low and the KSHV/HPV co-infection is seldom reported. Currently, it remains unclear about the role of KSHV co-infection in the development of HPV-related neoplasias. In this article, we have summarized the recent finding from clinic and bench indicating KSHV co-infection may represent a co-factor for the development of HPV-related carcinogenesis.

Keywords: KSHV, HPV, cervical cancer, oncogenic virus

Introduction

Certain subtypes of human papillomavirus (HPV) may cause warts on or around the female and male genital organs, which are called low-risk subtypes because they are seldom linked to cancer. In contrast, high-risk subtypes of HPV are strongly linked to several human cancers, including cervical, penile, anal and oral cancers [1, 2]. Among these, cervical cancer represents one of the most common malignancies in females worldwide. Although infection by HPV is the most important risk factor for cervical cancer, HPV infection is not the only cause of cervical cancer or not enough to initiate cervical cancer development, because most women with HPV infection do not get cervical cancer. In fact, certain other risk factors, like smoking and HIV infection, influence which women exposed to HPV are more likely to develop cervical cancer. In addition, certain other factors including co-infected pathogens, such as human immunodeficiency virus (HIV) and chlamydia, have been reported to increase the risk of women exposed to HPV for developing cervical cancer [3, 4].

Kaposi sarcoma-associated herpesvirus (KSHV, also known as human type 8 herpesvirus, HHV-8) represents a principal causative agent of several human cancers arising especially in those immunocompromised patients, including Kaposi's Sarcoma (KS), Primary effusion lymphoma (PEL) and Multicentric Castleman's disease (MCD) [5-7]. In fact, the immunosuppression (e.g., HIV infection, the use of immunosuppressive drugs) puts women at higher risk for HPV infection and cervical cancer development from precancerous conditions of the cervix. Published literatures have reported that KSHV DNA can be detected in the prostate, semen, oral cavity and the female genital tract [8-12]. KSHV can be transmitted via sexual contact including oral and anal sex, and via non-sexual routes, such as transfusion of contaminated blood and tissues transplants [13].

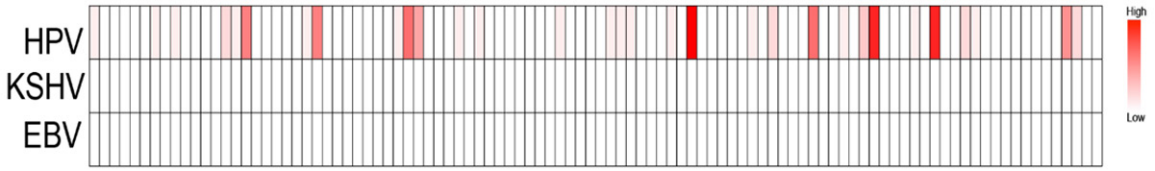

Figure 1. Detection of oncogenic viruses transcripts in the RNA-Seq datasets of cervical cancer samples. One hundred RNA-Seq cervical cancer datasets were obtained from the TCGA cohort and raw sequencing reads were analyzed as previously described [57]. Each vertical bar represents an individual patient and the color intensity reflects the levels of viral transcripts.

Moreover, the salivary transmission is thought to be as the main route of KSHV transmission, especially in children residing in endemic areas. Besides skin-to-skin contact, HPV can also be spread from one person to another through different sexual activities. Based on these common transmission routes, it is reasonable to speculate KSHV and HPV may have co-infection in some particular subpopulations, such as HIV+ individuals, organ transplant recipients. However, currently there are few studies reporting the co-infection of these two oncogenic viruses or their interaction in cervical samples and/or cervical cancer cells. It also remains unclear about the role of KSHV co-infection in the development of HPV-related cervical cancers. Here we have summarized the recent finding from our group and others in this interesting field and given some perspectives, too.

Epidemiology of KSHV and HPV co-infection in cervical samples

Like other herpesviruses, KSHV can also establish life-long latent infection in host cells with the expression of a limited number of viral genes. In contrast to the high prevalence of KSHV shedding in oral cavity, the detection rate of KSHV DNA or virus infection in cervical samples are relatively low or even totally negative in some studies. Whitby *et al* reported that KSHV DNA was detected in 3 of 11 cervical brush scrapes (CBS) obtained from KSHV-seropositive women attending the genitourinary medicine department [12]. In comparison, KSHV DNA was not detected in any of the 78 CBS from KSHV-seronegative women or in 96 CBS from women of unknown KSHV serostatus attending the colposcopy clinic. Another epidemiology study of KSHV infection in sex workers and women from the general population in Spain indicated that KSHV DNA was detected in 2% of the cervical samples of the prostitutes and in 1% of the cervical samples of women in the general population [14]. Moreover, they found that KSHV was more prevalent among HPV DNA-positive women (odds ratio = 2.5). Similarly, one study in 174 KSHV-seropositive female prostitutes in Mombasa, Kenya, showed that the prevalence of detection of KSHV was 4% in cervical swabs and 2.3% in vaginal swabs, although the status of HPV infection in these individuals remains unknown [15]. In contrast, one recent study found that HPV DNA was detected in 18/31 (58%) female genital brushings while none of these female genital brushings were KSHV DNA positive [16]. Another study reported that no cervical secretion from 112 Swedish women contained detectable KSHV DNA, although the antibodies to KSHV latent and lytic antigens were found in 2.7% and 24% of serum samples from the same group, respectively [17].

To detect the potential oncogenic pathogens in cervical cancer patient samples, a total of 100 RNA-Seq cervical cancer datasets were obtained from NIH The Cancer Genome Atlas (TCGA) cohort. Raw sequence data were aligned to a reference human genome (hg38; Genome Reference Consortium GRCH38) plus a library of virus sequences (including the sequences from all known human viruses documented by NCBI). We found that HPV transcripts were present in 31% of these samples but other oncogenic viruses including KSHV and EBV transcripts were not detectable (**Figure 1**). Furthermore, RNA-Seq datasets from a total of 27 cervical and/or endometrial cancer cell lines were downloaded from the NCBI Sequence Read Archive (SRA) and were then subjected to virome screening using the same informatics approach above. Our results show no evidence of KSHV and HPV co-infection in these tested cell lines. However, all these 100 RNA-Seq datasets were collected

from cervical cancer patients in the general non-HIV population, since no HIV reads were detected in these datasets. Actually, we cannot find any similar datasets from immunocompromised patients such as HIV+ individuals from TCGA cohort. As we know, the immunosuppression will greatly increase the chances of these oncogenic viruses co-infection.

Regulation of HPV oncogenic gene expression by KSHV co-infection in cervical cancer cells

High-risk HPV such as subtype 16 and 18 encoded E6 and E7 proteins are major viral oncoproteins which are closely associated with human cervical carcinogenesis [18]. E6 and E7 proteins can bind to the p53 and retinoblastoma (Rb) family proteins, respectively, resulting in the regulation of cell cycle and transformation [19]. Recent research has demonstrated E6 and E7 proteins can interact with or regulate many more cellular factors, including those proteins which regulate epigenetic marks and splicing changes in the cell, also contributing to oncogenesis [20]. Currently, it remains almost unclear how KSHV infection or KSHV-encoded proteins regulate HPV oncogenic gene expression in cervical cancer cells. Our recent studies have demonstrated that KSHV can successfully establish latent infection in a variety of HPV+ cervical cancer cell lines such as HeLa, SiHa and CaSki [21-23]. We also found that these viruses in latently infected cervical cancer cells possess normal replicative potential, since they can be induced into lytic phases by exogenous stimulus and finally produce new infectious particles [22].

Interestingly, our data indicated that KSHV infection significantly reduced both E6 and E7 expression from HPV16+ SiHa cells *in vitro* [22]. By using a cervical cancer xenograft model, we also confirm these results *in vivo* [23]. Furthermore, we found that LANA (Latency associated nuclear antigen) and vFLIP (viral FLICE inhibitory protein), two major KSHV-encoded latent proteins, responsible for the downregulation of E6 and E7 expression from SiHa cells [22]. Zhang *et al* have reported that interferon-β treatment induces one of cellular microRNAs, miRNA129-5p expression, while its levels gradually decrease with the development of cervical intraepithelial lesions and correlate with HPV E6 and E7 expression [24]. Following this discovery, we demonstrate that miRNA-129-5p is required for KSHV and/or viral latent proteins reducing E6 and E7 expression from SiHa cells [22]. Very interestingly, another group found that one of KSHV-encoded lytic protein, RTA (Replication and transcription activator), can bind to various HPV16 genomic regions and induce a significant upregulation of E7 transcription [25]. In fact, we also found that inducing lytic reactivation effectively impaired the reduction of E6 and E7 expression from KSHV-infected SiHa cells [22]. Therefore, these results indicate KSHV latent and lytic proteins may have distinct regulation of HPV oncogenic proteins expression in cervical cancer cells. Since KSHV is a big dsDNA virus with ~165 kb genome which containing 81 viral ORFs, as well as some microRNAs, non-coding RNAs, and a few small ORFs [26], it still requires a lot of work to understand how these viral components differentially regulate HPV oncogenic proteins expression in cervical cancer cells.

Regulation of cellular gene expression and functions by KSHV co-infection in cervical cancer cells

By using a cytokine/chemokine array, our recent study indicate that KSHV co-infection has increased several inflammatory factors production from SiHa cells, including Chemokine (C-X-C motif) ligand 1 (CXCL1), Interleukin 6 (IL-6), Plasminogen activator inhibitor-1 (PAI-1), Chemokine (C-C motif) ligand 5 (CCL5), Interleukin 8 (IL-8) and Macrophage migration inhibitory factor (MIF) [22]. Among these factors, CXCL1, its serum levels were significantly higher in patients with cervical squamous cell carcinoma (CSCC) when compared with patients with cervical intraepithelial neoplasia (CIN) and the healthy controls [27]. IL-6 has been found to promote cervical tumor growth via vascular endothelial growth factor (VEGF)-dependent angiogenesis or by modulating the apoptosis threshold [28, 29]. Interestingly, a recent meta-analysis study indicates that the single-nucleotide polymorphisms (SNP) of IL-6 (rs1800795) is associated with cervical cancer risk [30]. Another upregulated factor, PAI-1, an inhibitor of urokinase-type plasminogen activator, has been found with increased expression in cervical tumor tissue, specifically in aggressive tumors [31]. Moreover, targeting PAI-1 expression or function results in the reduction

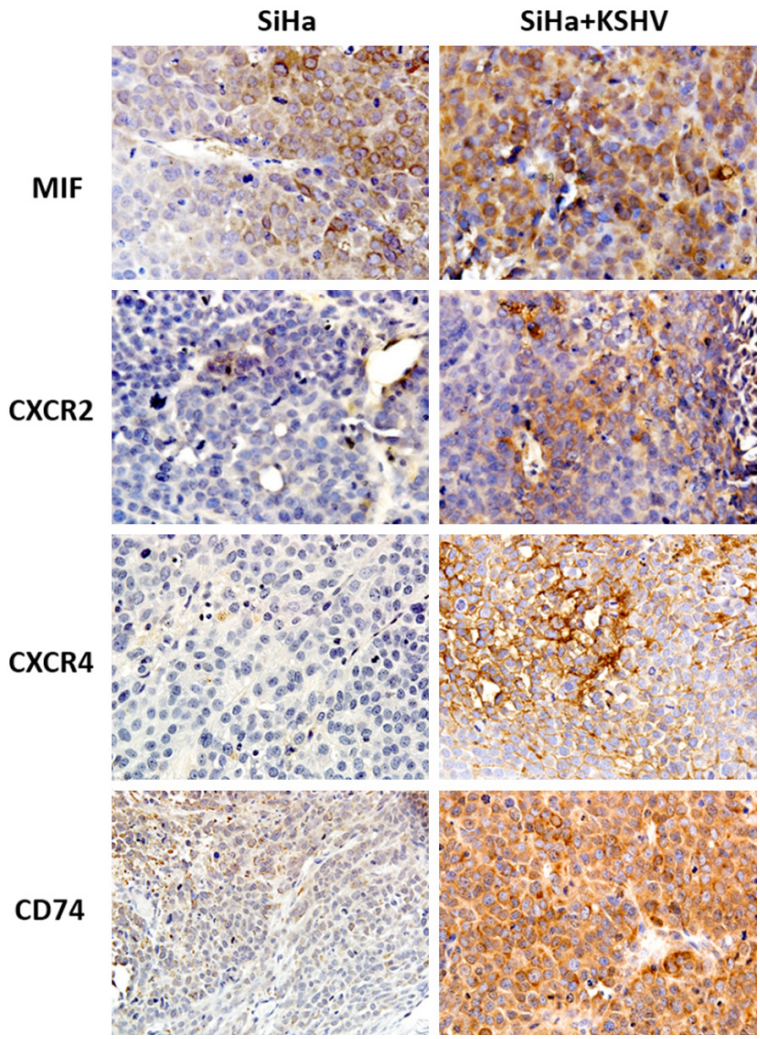

Figure 2. The upregulation of MIF and its receptors by KSHV co-infection in HPV+ cervical cancer tissues. The mock or KSHV co-infected SiHa cells (approximately 5 × 10^5 cells were mixed at a ratio of 1:1 with growth factor-depleted Matrigel) were injected subcutaneously into the right flanks of nude mice, respectively. The mice were observed and measured every 2~3 d for the presence of palpable tumors for ~40 d. Protein expression within tumor tissues from representative injected mice was measured by using immunohistochemistry staining.

of cellular proliferation, cell adhesion, colony formation, while the induction of apoptosis and anoikis in cervical cancer cells [32].

One of these factors, MIF, is well recognized as a cancer biomarker protein, since its expression in normal cells is several orders of magnitude lower than levels observed in cancer cells [33-36]. By using ELISA, we recently have found that KSHV co-infection significantly increases MIF secretion from HPV+ cancer cell lines such as SiHa and CaSki (5-8 folds increasing) [23]. Soluble MIF produced by cancer cells is imported into the cytoplasm and nucleus of its target cancer cells via an autocrine loop [37, 38]. MIF enters target cells by binding to its cellular receptors such as CXCR2, CXCR4 or CD74 [38, 39]. Our recent *in vivo* study indicates that the significant upregulation of MIF and its receptors CXCR2, CXCR4 and CD74 in tumor tissues from KSHV co-infected SiHa injected mice when compared to those from SiHa injected mice (**Figure 2**). In fact, one previous study reported the overexpression of MIF in invasive cervical cancer samples when compared to cervical dysplasias samples [40]. Another study also found that MIF and CD74 expression was significantly higher in CIN or CSCC than in the normal samples [41]. The overexpression of MIF was correlated with deep stromal infiltration, and both MIF and CD74 protein levels were associated with microvessel density [41]. Our recent findings suppose KSHV co-infection may represent one novel mechanism to upregulate MIF and its receptors from cervical cancer cells. Targeting MIF effectively inhibits cervical cancer cell growth, migration, invasion, colony formation and tumorigenesis *in vitro* and *in vivo* [42-44]. Moreover, one recent study indicates that MIF polymorphisms (-794CATT$_{5\text{-}7}$) can be used as a potential biomarker for early-stage cervical cancer [45].

Conclusion and prospective

Currently, there are limited data about the co-infection of KSHV and HPV in cervical samples and/or cervical cancer cells. However, recent findings from both *in vitro* and *in vivo* studies indicate that KSHV may act as one of co-factors for HPV-related cervical carcinogenesis (especially in those immunocompromised patients),

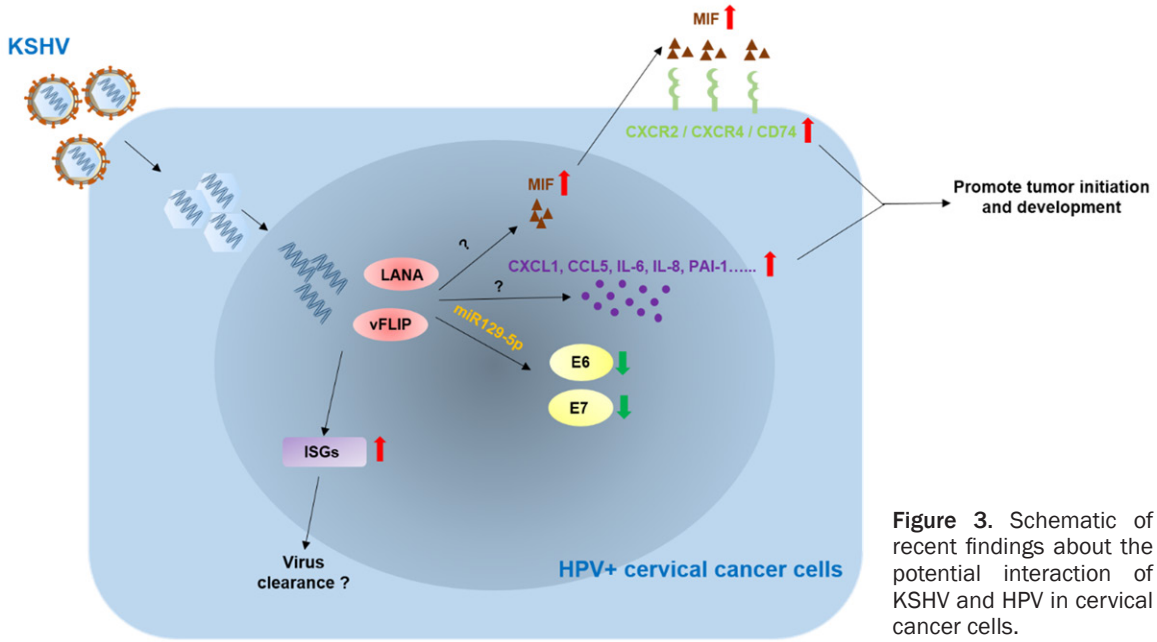

Figure 3. Schematic of recent findings about the potential interaction of KSHV and HPV in cervical cancer cells.

although there are still a lot of remaining questions need to be further investigated:

1) Low detection rate of KSHV shedding as well as of KSHV/HPV co-infection in cervical samples and/or cervical cancer cells. Although cervical cancer cells or other mucosa epithelial cells have been shown fully susceptible to KSHV infection, the detection rate of KSHV (viral DNA) is very low in cervical benign and malignant samples. We think one of reasons is that the upregulated inflammatory cytokines/chemokines as well as Interferon (IFN)-induced genes by KSHV co-infection [22] may promote the recruitment of immune cells, enhance local inflammatory response, and finally facilitate attacking infected cells and/or the clearance of KSHV (especially in the immunocompetent patients). Another possible reason is that there is low number of cervical cells are latently infected by KSHV in most of patients which causing difficultly acquired by cervical biopsy. Finally, the sensitivity and accuracy of current methods for detection of KSHV still need to be improved.

2) Downregulation of E6 and E7 expression by KSHV co-infection of cervical cancer cells. Although these were found from cervical cancer cell lines or xenograft models [22, 23], the underlying mechanisms remain largely unclear. In spite of hijacking these HPV-encoded major oncogenic proteins expression, KSHV co-infection can maintain cervical cancer cells malignant behaviors, such as invasion, colony formation and tumorigenesis in animal models, which are through the manipulation of some certain cellular genes functions such as MIF and its signaling [23]. Therefore, KSHV co-infection may cause some HPV-independent factors contributing to cervical carcinogenesis (summarized in **Figure 3**).

3) Regulation of KSHV infection or viral protein functions by HPVs. On the other hand, we almost do not know whether HPVs including those different subtypes are able to affect KSHV infection of cervical epithelial cells, viral latency/lytic reactivation, virus replication, etc. These may represent an interesting direction for future investigation.

4) KSHV and HPV interaction in other cancers. Besides cervical cancers, these two oncogenic viruses co-infection or interaction may exist in other types of cancer. For example, high-risk HPV infection is also the etiological agent of some oral and oropharyngeal cancers [46-48]. As we know, oral cavity represents the major reservoir of KSHV and exchange of oropharyngeal secretions is an important route for this virus transmission. Interestingly, one recent study indicates that KSHV is similarly detectable across all levels of CD4 counts in HIV+

patients [49]. In addition, oral cavity involvement represents the initial manifestation of KS in 20-60% of HIV-associated cases, with the involvement of the oral cavity ultimately seen in the majority of patients [50-52]. High-risk HPVs are also closely related to anal cancer particularly in HIV+ men having sex with men (MSM) [53, 54], this subpopulation usually having high prevalence of KSHV infection [55, 56]. Therefore, it will be interesting to explore and determine whether KSHV/HPV interaction plays some roles in the development of other cancers in future studies.

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Disclosure of conflict of interest

None.

Abbreviations

HPV, human papillomavirus; HIV, human immunodeficiency virus; KSHV, Kaposi sarcoma-associated herpesvirus; KS, Kaposi's Sarcoma; PEL, Primary effusion lymphoma; MCD, Multicentric Castleman's disease; CBS, cervical brush scrapes; LANA, Latency associated nuclear antigen; vFLIP, viral FLICE inhibitory protein; ORF, Open reading frame; CXCL1, Chemokine (C-X-C motif) ligand 1; IL-6, Interleukin 6; PAI-1, Plasminogen activator inhibitor-1; CCL5, Chemokine (C-C motif) ligand 5; IL-8, Interleukin 8; MIF, Macrophage migration inhibitory factor; CSCC, cervical squamous cell carcinoma; CIN, cervical intraepithelial neoplasia; VEGF, vascular endothelial growth factor; SNP, single-nucleotide polymorphisms; IFN, Interferon; MSM, men having sex with men; TCGA, The Cancer Genome Atlas; SRA, Sequence Read Archive; NCBI, National Center for Biotechnology Information; ISGs, Interferon stimulated genes.

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Oncogenesis

REVIEW ARTICLE Open Access

Transactivation of human endogenous retroviruses by tumor viruses and their functions in virus-associated malignancies

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Abstract

Human endogenous retroviruses (HERVs), viral-associated sequences, are normal components of the human genome and account for 8–9% of our genome. These original provirus sequences can be transactivated to produce functional products. Several reactivated HERVs have been implicated in cancers and autoimmune diseases. An emerging body of literature supports a potential role of reactivated HERVs in viral diseases, in particular viral-associated neoplasms. Demystifying studies on the mechanism(s) of HERV reactivation could provide a new framework for the development of treatment and prevention strategies targeting virus-associated tumors. Although available data suggest that co-infection by other viruses, such as Kaposi's Sarcoma-associated herpesvirus (KSHV) and Epstein–Barr virus (EBV), may be a crucial driving force to transactivate HERV boom, the mechanisms of action of viral infection-induced HERV transactivation and the contributions of HERVs to viral oncogenesis warrant further studies. Here, we review viral co-infection contributes to HERVs transactivation with focus on human viral infection associated oncogenesis and diseases, including the abilities of viral regulators involved in HERV reactivation, and physiological effects of viral infection response on HERV reactivation.

Background

Human endogenous retroviruses (HERVs) are a subgroup of retroviruses integrating their sequences into host genome after exogenous retrovirus infection millions of years ago, which account for about 8–9% of human genome[1,2]. Due to the accumulation of mutation, most HERVs are commonly inactive and unable to replicate. However, some HERVs still have open reading frames and keep a potential for protein expression[3,4]. A growing number of findings suggest that viral products of HERVs may have a role in species evolution, as well as various diseases[3,5–7].

Retroviruses are double-stranded positive-sense RNA viruses encoding and carrying reverse transcriptase (RT) to reversely transcribe RNAs to DNAs. These viral DNAs are then integrated into the host DNA mediated by its integrase enzyme (IN), thus creating a provirus, which can translate and transcribe viral products[8,9]. Similar to integrated retrovirus, a complete sequence of HERVs are mainly composed of *gag*, *pro*, *pol*, and *env* regions sandwiched between two long terminal repeats (LTRs) (Fig. 1). LTRs contain main promoters, enhancers, and transactivation regions for HERV transcription, thus regulating activation and expression of HERV genes[10]. The *gag* and *pol* usually encode polyproteins, which are then processed into individual proteins. The products of *gag* are structural proteins and *pol* codes for the RT, IN, and RNAse H. Notably, unlike HIV genome, the *pro* gene of HERVs is separated from the *pol* reading frame. The product of *env* gene is a glycosylated protein and is cleaved into two viral

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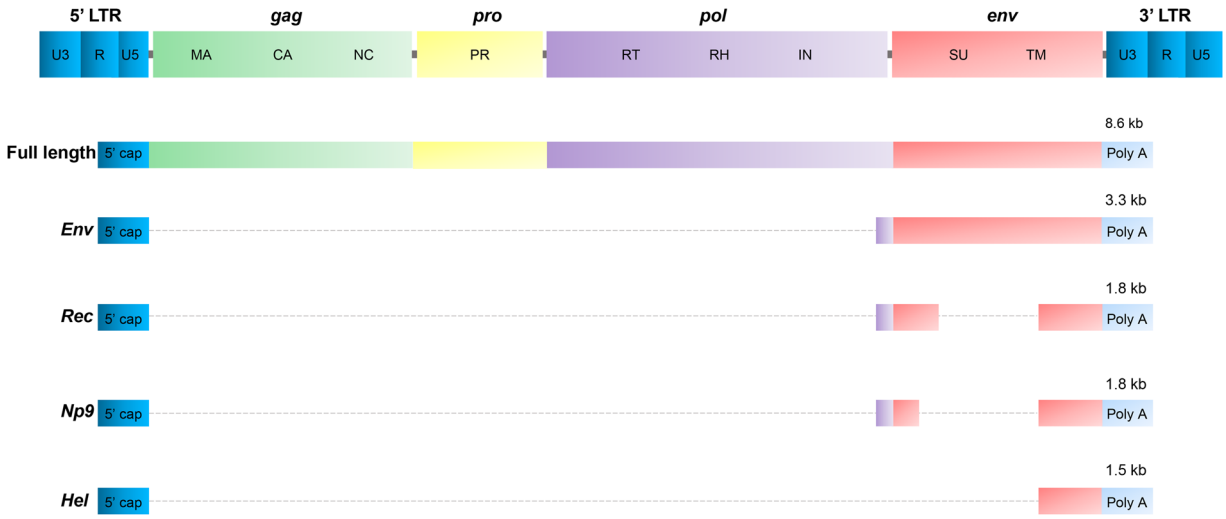

Fig. 1 Diagrams of HERV-K proviruses and their transcripts. A compete sequence of HERVs are composed of *gag*, *pol*, *pro*, and *env* regions sandwiched between two long terminal repeats (LTRs). *Gag* encodes the structural components of matrix (MA), capsid (CA), and nucleocapsid (NC). The products of *pol* gene are reverse transcriptase (RT), integrase (IN), and RNase H (RH). The *pro* mainly encodes the enzyme protease (PR), while *env* encodes Env surface (SU) and transmembrane (TM) subunits. LTRs are composed of U5 region, U3 region and repeat sequences (R). The HERV-K (HML-2) usually expresses a full-length transcript (8.6-kb) and encodes the gag, pro, and pol polyproteins. *Env* gene transcripts two singly spliced products, a 3.3-kb product to encode Env polyprotein, and a 1.5-kb product of unknown function known as the *hel* transcript, and a doubly spliced product (1.8-kb) to encode either the Rec or Np9 accessory proteins depending on the presence or absence of a 292-bp deletion at the *pol*/*env* boundary

envelope proteins, a surface unit (SU) and a transmembrane unit (TM)[3,11,12].

Currently, HERVs are classified into 22 independently acquired families based on the first-letter amino-acid core of the tRNA of the primary binding site used by HERV to start reverse transcription[13]. Of these, some HERV families, such as HERV-K, were identified to be relevant with the development of human cancers, such as breast cancer[14], lung cancer[15], prostate cancer[16], hepatocellular carcinoma (HCC)[17], melanomas[18], germ cell tumor[19], leukemia[20] and, lymphoma[21]. HERV-K is the most recent HERV family acquired by humans at around three million years ago[22]. In contrast to all other HERVs, some HERV-Ks are to date the only known human endogenous proviruses that have retained open reading frames for all viral proteins, such as HERV-K (HML-2)[8,23]. HERV-Ks are formed by 11 subgroups (HML-1-HML-11), with the most-studied one in cancers being HERV-K (HML-2)[8]. HERV-K (HML-2) has two major types of proviruses (type I and II), for which the nomenclature is based on the presence (type I) or absence (type II) of a 292-bp deletion at the *pol*/*env* boundary encoding two variant proteins, Np9 and Rec, respectively[11,24]. The type II provirus produces the regulatory protein Rec by a singly spliced transcript, while the type I provirus produces Np9 through a doubly spliced transcript in the *pol*/*env* boundary region. HML-2 also expresses a 1.5-kb transcript with unknown function referred to as the *hel* transcript[11,12] (Fig. 1). Furthermore, both Rec and Np9 have been reported as oncogenic proteins and are present in a variety of tumors and transformed cells.

Although the precise role of HERVs in development of tumors has not been fully elucidated, there are increasing data suggesting that HERVs are closely related to human malignancies. Many studies have identified high levels of the expressed products of HERVs in cells, tissues, and blood of patients with cancers[14–22]. The transactivation of HERVs may affect carcinogenesis process through directly expressing viral mRNA, functional proteins, and/or viral particles, or indirectly activating tumor-associated genes. Viral products of many HERVs, such as the K, H, R, and T families, have been detected in cells, blood and tissues of patients with lung cancer or breast cancers. Levels of HERVs transactivation have been shown to be much higher in these patients than those in healthy volunteers[25–27]. The positive correlation of HERVs transactivation with cancer is strongly supported by the observation that some specific antibodies or shRNAs against HERV-K possess inhibitory effect on the growth of cancer cells in vitro and in vivo[28,29]. Thus, HERVs could be considered as suitable prognostic markers for a variety of malignant diseases, such as lung cancers and HCC[17,25]. Additional studies have found that Np9 and Rec proteins of HERV-K physically and functionally interact with the promyelocytic leukemia zinc finger (PLZF) tumor suppressor to regulate cancer cell proliferation and survival

Table 1 Viral infections induced HERVs transactivation

Viruses	HERV family	Possible mechanisms	Ref.
HSV-1	W, K	IE1 stimulates LTR of HERV-W trough enhancing the activity of Oct-1; ICP0 increases transcription LTR of HERV-K through AP-1 site.	36,42,43
VZV	Unknown	VZV can sustain the increase in the RT expression.	89
HCMV	T, W, F, K, L	HCMV-induced cytokines and growth factors may enhance HERV activation.	68,69
EBV	W, K	LAM-2A and LMP-1 activate HERV-K in infected B lymphocytes; EBV infection activates HERV-K in resting B lymphocytes through binding CD21; HERV-W activation was regulated by EBV gp350 in PBMC.	37,57–59,90
HHV-6	K	HHV-6A induces HERV-K18-encoded superantigen through IFN-α; HHV-6B induced superantigen HERV-K18, which may have consequences for the development of autoimmunity.	91,92
KSHV	K	LANA induces env transcripts through enhancing ERK activity; vFLIP induces env transcripts through activating NF-κB activity.	33
HIV-1	K, E, W, T	HERV-K (HML-2) is activated by Tat through regulating NF-κB and NF-AT.	2,32,40
HTLV-1	K, E, W, H	Tax is able to activate HERV LTRs, mainly of HERV-W and -H.	35,74
HBV	W	HBV X Protein induces overexpression of HERV-W env through NF-κB.	34
Influenza A virus	W	Influenza A virus infection can transactivate ERVWE1 by increasing the transcription of GCM1 and reducing the repressive histone mark H3K9me3.	36,41

through altering the expression of the c-*Myc* proto-oncogene[30,31].

Viral infection and HERVs transactivation

Although the detailed mechanisms of HERVs transactivation remain largely unclear, a variety of inducers have been reported, including some external and internal signals. Of these, viral infection plays important roles in the regulation of HERVs transactivation (Table 1). Many recent studies have shown that infection with exogenous viruses, such as HIV-1, HBV, HTLV-1, Influenza A virus, and herpesviruses, can induce significant HERVs transactivation, which in turn, co-contributes to the development of viral diseases, including virus-associated tumors[32–37]. For instance, many HERVs are activated in HIV-1-infected patients, and the levels of HERV products are decreased in patients with anti-HIV treatment[38]. Studies on mechanisms of HERV-K transactivation show that HIV-1 Tat protein can induce HERV-K expression through regulating the NF-κB and NF-AT pathways[39,40]. Also, Influenza A/WAN/33 virus infection can induce transcriptional de-repression of the ERVWE1 of HERV-W by increasing transcription of GCM1 and reducing H3K9me3[41]. Another example is that of Herpes simplex virus 1 (HSV-1) infections, which can activate both HERV-W and HERV-K through two different pathways, in which viral IE1 enhances the activity of Oct-1 to stimulate HERV-W[42], while ICP0 upregulates the activity of AP-1 to activate HERV-K[43]. In fact, ~20% of human cancers have been found to be related to viral infections, but the mechanism of viral oncogenesis is largely unclear. However, recent data about HERVs transactivation induced by tumor viruses and their function in malignant diseases indicate that HERVs transactivation may act as potential regulators or co-contributors to viral oncogenesis. Here, we present a summary of recent findings regarding the relationship of different tumor viruses with HERVs transactivation.

Tumor virus infections and HERVs transactivation

KSHV infection and HERVs transactivation

Kaposi's sarcoma-associated herpesvirus (KSHV) is a double-strand DNA virus classified as a type 8 member of human herpesvirus family (HHV-8)[44]. Previous studies have confirmed that KSHV infection is capable of causing Multicentric Castleman's disease (MCD) and several cancers, such as Kaposi's sarcoma (KS), and primary effusion lymphoma (PEL)[45]. KSHV-induced KS is one of the most common acquired immuno-deficiency syndrome (AIDS)-associated tumors. Despite recent progress in the development of treatments for KSHV-associated malignancies, more effective therapies remain urgently needed.

KSHV infection has two alternative life cycle programs, latent and lytic phases, both of which can contribute to the development of KSHV-induced cancers[46]. Generally, latent infection is established and persists in host cells following KSHV de novo infection, with only a small

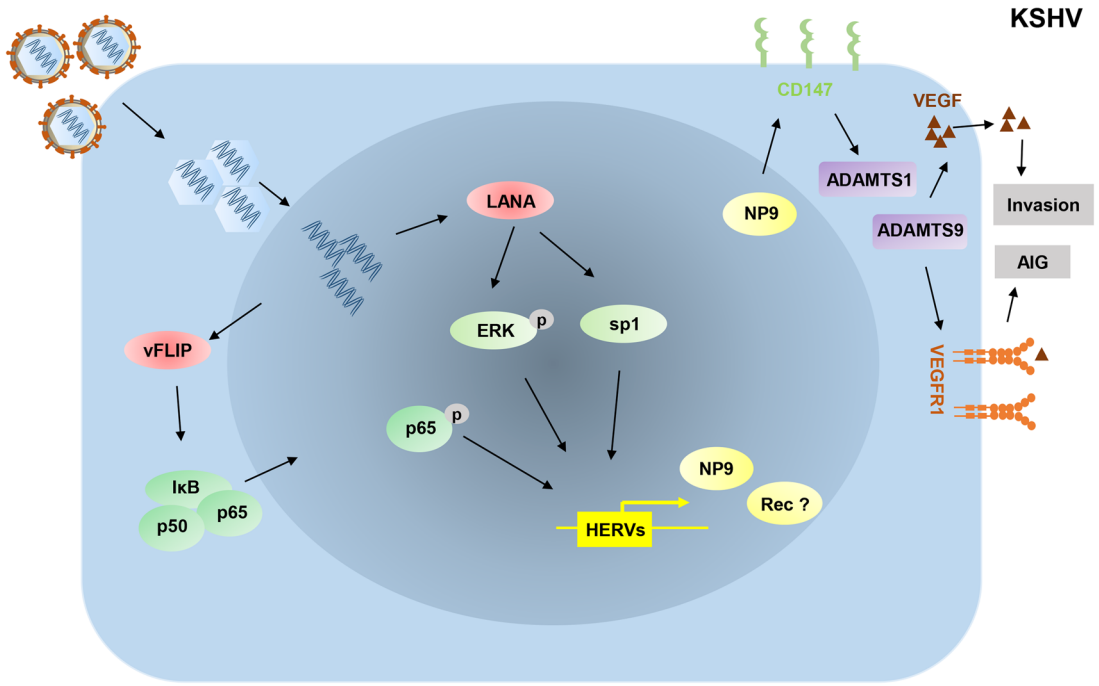

Fig. 2 Schematic diagram of potential mechanisms for KSHV promoting HERV transactivation. During KSHV de novo infection, LANA induces *env* transcripts through enhancing ERK activity, and vFLIP induces *env* transcripts through activating NF-κB activity. Np9 expression mediated by KSHV can promote virus-induced anchorage-independent growth (AIG) and invasion through the CD147-ADAMTS1/ADAMTS9-VEGF/VEGFR1 axis. LANA: a latency-associated nuclear antigen; vFLIP: viral FADD-like interleukin-1-β-converting enzyme (FLICE)/caspase-8-inhibitory protein; ERK: extracellular-signal-regulated kinase; ADAMTS1: a disintegrin and metalloproteinase with thrombospondin motifs 1; ADAMTS9: a disintegrin and metalloproteinase with thrombospondin motifs 9; VEGF: vascular endothelial growth factor; VEGFR1: vascular endothelial growth factor receptor 1

population of cells undergoing spontaneous lytic replication in a temporally ordered manner. During latency, only a limited number of latent genes, such as *ORF71* (*v-FLIP*), *ORF72* (*v-Cyclin*), *ORF73* (*LANA*), *K12* (*Kaposin*), and viral miRNAs are constitutively expressed to be involved not only in the maintenance of viral genome stabilization, but also in the regulation of host microenvironment. Of the latent gene products, LANA and v-FLIP play critical roles in viral pathogenesis, especially KSHV-induced tumorigenicity[47].

KSHV-induced tumors are found most frequently in HIV-1-infected or other immunosuppressed patients[48]. HERVs have also been associated with HIV-1-infected and autoimmune diseases[6,32]. Thus, these data hint the potential relevance of KSHV infection with HERVs expression. Interestingly, the hypothesis is supported by the observation that the high levels of HERV-K (HML-2) *env* transcripts has been found in peripheral blood mononuclear cell (PBMC) from KSHV-infected HIV + patients[33]. Although HIV-1 is one of the viral factors inducing HERVs transactivation and HIV-1 Tat promotes expression of HERVs transcripts through regulating NF-κB and NF-AT signals, the level of HERV *env* transcripts are much lower in PBMC from HIV + patients without KSHV co-infection, suggesting that KSHV is also an activator or co-factor of HERVs transactivation.

Additional experimental data support that HERV-K (HML-2) transactivation is closely related to KSHV infection. The significantly higher levels of transcriptional products of HERV-K (HML-2) are found in KSHV + PEL tumor cells and KSHV de novo infected endothelial cells when compared to virus-negative control cells[33]. However, the levels of HERV-K associated transcripts are almost not changed in UV-inactivated KSHV-infected cells, implying HERV-K transactivation by KSHV infection may require the expression of KSHV latent transcripts. Mechanistic studies on KSHV-activated HERV-K (HML-2) show that two viral latent proteins, LANA and v-FLIP, regulate the transcription of HERV-K through both classical intracellular signaling pathways and cellular transcriptional factors (Fig. 2). LANA induces HERV-K *env* transcription through enhancing ERK signaling activity[33]. Furthermore, LANA may regulate HERV-K LTRs, which contain potential binding sites for viral and cellular transcriptional factors, through directly interacting with Sp1, a classical modulator of HERV-K LTR activities[33]. In fact, some other mechanisms, including DNA methylation, histone modification and the Rb

(retinoblastoma) pathway, are also involved in the regulation of HERV-K transactivation[33]. Interestingly, LANA has been found to interact with or regulate Rb/E2F pathway and many epigenetic factors, such as EZH2, KDM3a, and DNMT3a[49,50]. Therefore, these additional mechanisms need to be further investigated. HERV-K *env* transcripts are also upregulated by another KSHV-encoded latent protein, v-FLIP, potentially through the activation of NF-κB pathway[33].

HERV-K *env* transcripts encode two oncogenic proteins, Rec and Np9, both of which can promote cancer development. However, more prominent expression of Np9 than Rec has been found in KSHV-infected cells and AIDS-KS tumor tissues[33]. Moreover, Np9 is closely related to KSHV-induced invasion and anchorage-independent growth of primary endothelial cells through the regulation of the CD147-ADAMTS1/ADAMTS9-VEGE/VEGFR1 axis, enhancing viral pathogenesis in infected cells. Interestingly, silencing Np9 by RNAi in KSHV-infected TIVE-LTC cells dramatically reduced cell growth in vitro and suppressed the formation of KSHV-induced tumors in nude mice, suggesting that Np9 protein is an important co-factor for KSHV-induced tumorigenesis[33]. Therefore, the detailed function of HERVs transactivation in KSHV-related cancer progression, which may represent a promising direction for developing targeted therapy for KSHV-associated malignancies, needs to be further investigated.

EBV infection and HERVs transactivation

Epstein–Barr virus (EBV), the type 4 member of the HHV family, is a ubiquitous virus. Studies show that up to 95% of all adults in the world have antibodies against this virus[51]. Previous studies confirmed that EBV infection has been linked to a number of malignant diseases, such as infectious mononucleosis, Burkitt's lymphoma, Hodgkin's lymphoma, naso-pharyngeal cancer, NK/T-cell lymphoma, post-transplant lymphoma, and multiple sclerosis[44].

Similar to other herpesviruses, EBV infection has two alternative life cycle programs, latent and lytic phases[52]. While the lytic replication of EBV is pivotal to viral transmission and genome maintenance, the latency makes a more direct contribution to lymphoproliferative diseases[53]. EBV latent infection is established and persists in B cells and epithelial cells, however different latency programs are possible in these two types of cells. Based on which latent genes are expressed, latency of EBV can be divided into three distinct stages, Latency I, II, or III[54]. The latent gene products mainly include Epstein–Barr nuclear antigen 1 (EBNA1)/EBNA2/EBNA3A, EBNA3B, and EBNA3C, latent membrane protein 1 (LMP-1)/LMP-2A and LMP-2B, nuclear antigen leader protein, and virus-encoded small RNAs (EBERs), all of which are involved in the regulation of host gene expression and viral pathogenesis[53].

EBV infection usually induces superantigens (SAgs)-activated T-cell immune response[55]. T-cell activation mediated by SAgs plays important roles in viral maintenance and the development of virus-associated diseases[55,56]. Interestingly, Sutkowski et al. found that EBV infection transactivates the expression of HERV-K18 *env* gene that possesses SAg activity, which was further demonstrated by MHC class II dependent preferential activation of TCRVB13 T cells in response to murine B cells transfected with the HERV-K18 *env* gene[37]. Further studies revealed that EBV transactivates the HERV-K18 SAg through viral latent protein LMP-2A, LMP-1, and its cellular receptor, CD21[57,58]. While LMP-2A and LMP-1 each contribute to the induction of the SAg activity of HERV-K18 *env* gene in latently infected cells in vitro, EBV-encoded gp350 protein also triggers the expression of HERV-K18 *env* gene in resting B cells through binding to human CD21[58]. Data show that the immunoreceptor tyrosine-based activation motif (ITAM) of LMP-2A is important for HERV-K18 *env* transactivation through CrKL pathway[57]. The activation of ERK and NF-κB pathways may be important steps in LMP-1-mediated HERV-K activation, whereas gp350 activates HERV-K through protein kinase C, protein tyrosine kinase, and NF-κB pathways[58] (Fig. 3). A recent study found that EBV-encoded gp350 also activates HERV-W/syncytin-1 in cells derived from blood and brain through the NF-κB pathway or some pro-inflammatory cytokines[59], implying that HERV-W may be a potent contributor involved in the pathogenesis of multiple sclerosis.

Interestingly, HERV-K transactivation induced by EBV infection may in turn regulate viral gene expression. One study shows that HERV-K Np9 is strongly upregulated in EBV-transformed lymphocytes and is detected in many EBV + tumor cells[60]. These data show that Np9 protein is able to hijack EBNA2 to reduce the binding ability of EBNA2 to DNA-bound RBP-Jκ leading to the down-regulation of the EBNA2-mediated activation of the viral C- and LMP-2A promoters[60] (Fig. 3). Inhibitory effect of EBV-induced Np9 on viral gene expression may represent a protective mechanism, which controls excessive expression of viral products to promote proliferation of infected cells.

HCMV infection and HERVs transactivation

Human cytomegalovirus (HCMV), a double-strand DNA virus, belongs to HHV family (also known as human herpesvirus-5, HHV-5)[61]. HCMV remains in latent phase within the body throughout life following primary infection, but it can be reactivated at any time[62]. HCMV infection is typically unnoticed in healthy people, but may cause life-threatening diseases in

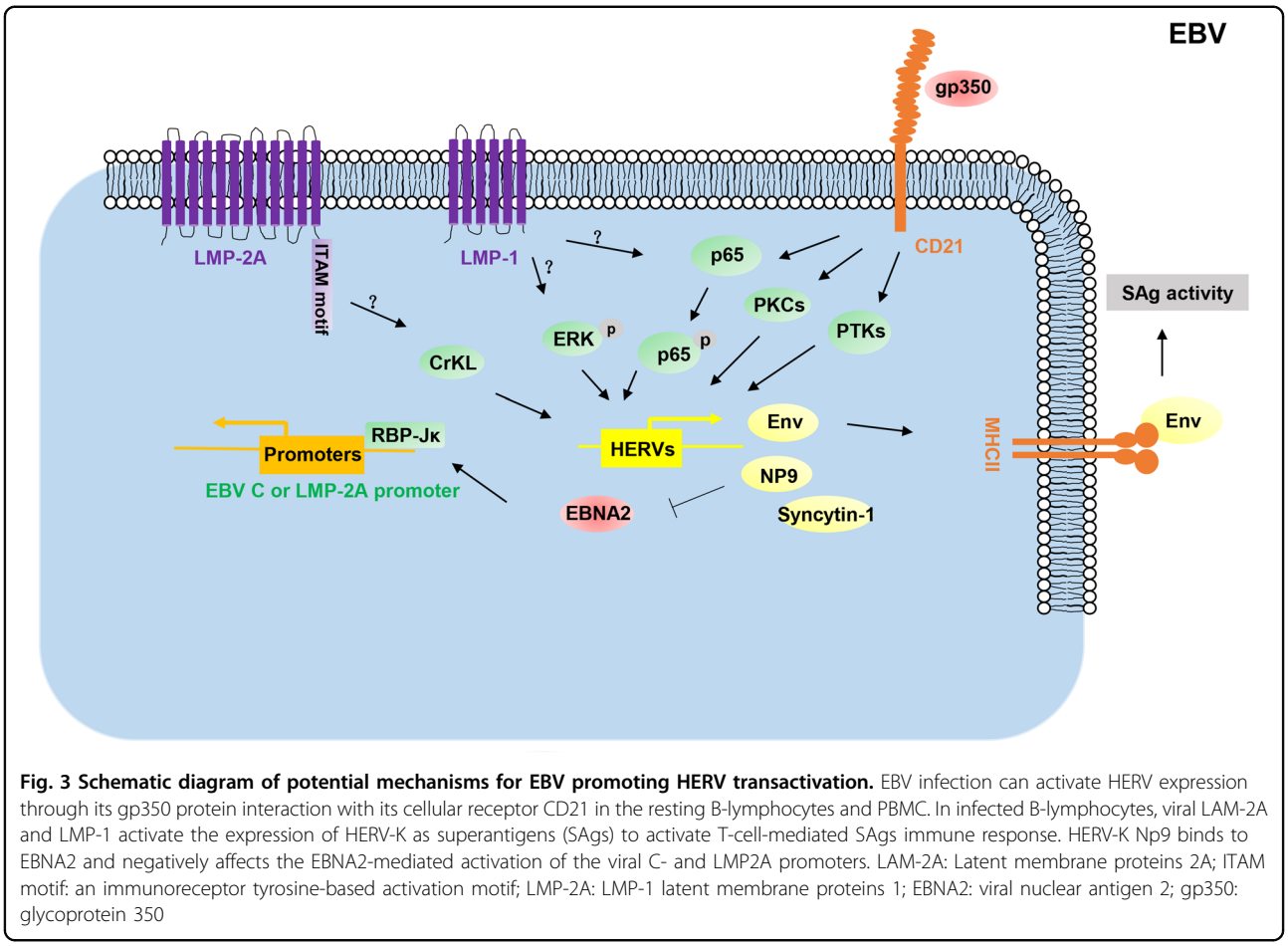

Fig. 3 Schematic diagram of potential mechanisms for EBV promoting HERV transactivation. EBV infection can activate HERV expression through its gp350 protein interaction with its cellular receptor CD21 in the resting B-lymphocytes and PBMC. In infected B-lymphocytes, viral LAM-2A and LMP-1 activate the expression of HERV-K as superantigens (SAgs) to activate T-cell-mediated SAgs immune response. HERV-K Np9 binds to EBNA2 and negatively affects the EBNA2-mediated activation of the viral C- and LMP2A promoters. LAM-2A: Latent membrane proteins 2A; ITAM motif: an immunoreceptor tyrosine-based activation motif; LMP-2A: LMP-1 latent membrane proteins 1; EBNA2: viral nuclear antigen 2; gp350: glycoprotein 350

immunocompromised hosts, such as HIV-infected persons, organ transplant recipients, or newborn infants[63]. Increasing data show that HCMV may possess oncogenic or onco-modulatory functions in human cancers, because of its high prevalence in cancers of different origin, such as glioblastoma, medulloblastoma, neuroblastoma, colon, breast, and prostate cancers, and its ability to control the expression of host genes, especially the activation of cellular oncogenes and inhibition of tumor suppressor genes[64–67].

Recent studies have revealed that in GliNS1 cells, HCMV infection regulates the transactivation of HERV-T, HERV-W, HERV-F, ERV-9, HERV-K (HML-2, -3, -4, -7, and -8 groups), and HERV-L groups, and in HUVEC cells from healthy donors, ERV-9, HERV-F, and HERV-K (HML-2, -5, and -6 groups) were upregulated under HCMV infection condition[68]. Furthermore, in kidney transplant recipients, HCMV induces HERV-K and HERV-W expression, demonstrating its clinical relevance[69]. However, lytic replication of HCMV may not be the reason of HERV-K expression due to no inhibitory effect of blocking HCMV replication by ganciclovir or silencing of IE1/IE2 on HERV-K transactivation[68]. Interestingly, in contrast to KSHV, UV-inactivated HCMV still activates HERV-K expression, but the increase in the HERV-K activity is far less pronounced than in normal HCMV infection[68]. Thus, activation of HERVs by HCMV infection may be directly induced through some cytokines and/or growth factors in response to viral infection.

HTLV-1 infection and HERVs transactivation

Human T-lymphotropic virus 1 (HTLV-1) belongs to a group of human retroviruses and is known as the causative agent in adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)[70]. Although most HTLV-1 infected patients maybe asymptomatic throughout their lives, this virus is now estimated to infect 5–10 million people worldwide[71]. The viral Tax protein has been considered to play an important role in the development of HTLV-1-associated diseases. HTLV-1 Tax protein performs the powerful function of activator to modulate the expression of many viral and cellular genes, such as CREB, NF-κB, and SRF[72,73].

It has been found that HTLV-1 Tax protein activates LTR of several HERVs, including HERV-W, HERV-H, HERV-E, and HERV-K families, speculating a potential link between HERVs transactivation and HTLV-1-associated diseases[35]. Moreover, the data from HLTV-1 infected patients show an increased prevalence of antibodies to Pol and Gag peptides of the retrovirus HERV-K10, homologous to HTLV-1 gp21 envelope and p24 Gap protein, respectively[74]. This prevalence was observed to be higher in HLTV-1 infected patients with myelopathy (87%) vs non-myelopathy (5.2%)[74]. Thus, HTLV-1 Tax-activated HERVs and/or HTLV-1-induced immuno-cross-reactivity may be involved in the pathogenesis of these virus-associated diseases.

HBV infection and HERVs transactivation

Hepatitis B virus (HBV), a small double-stranded DNA virus, causes acute and chronic hepatitis B in humans[75]. Chronic hepatitis B caused by HBV infection is the major cause of HCC worldwide, and remains therefore a major public health problem globally[75,76]. HBV-encoded X protein (HBx) is believed to be a potent regulator in the pathogenesis of HBV-related HCC[77].

HBx is a multifunctional oncogenic protein that modulates and activates the expression of many viral and cellular factors[77]. A recent study showed that HBx increased the promoter activity of HERV-W *env* to up-regulate its expression through the NF-κB pathway in human hepatoma HepG2 cells[34]. However, elucidating the function of HERVs transactivation in HBV-induced HCC still requires further investigation. Although the association of HERVs transactivation with HBV-induced HCC remains largely unclear, an interesting study showed that HERV-K transactivation is correlated with the prognosis and progress of HCC[17]. These data may provide a new insight about HERVs transactivation in HBV-associated HCC development.

Conclusion

In contrast to other "conventional" cancers, the role of HERVs transactivation in viral oncogenesis remains largely unknown. In recent years, the mechanisms of tumor virus-induced HERVs transactivation have been partially explored: (1) Virus-mediated transcriptional factors—the LTR regions of HERVs carry binding sites for many transcriptional factors (e.g., NF-κB), which can be activated by viral products and result in the induction of HERVs gene expression[78,79]. For example, KSHV LANA, EBV LMP-1, and HBV HBx can induce HERV transactivation through the NF-κB signaling pathway[33,34,57]. HLTV-1 Tax is also a classical activator of gene expression through modulating NF-κB activity, contributing to Tax-induced HERVs transactivation[35]. (2) Viral products directly regulate HERVs transactivation—many viral products can bind to the promoters of viral or host genes to regulate gene expression as transcriptional factors, such as EBV EBNAs and KSHV LANA[80,81]. Therefore, these viral products may directly bind to the LTR regions of HERVs to mediate their transactivation, although these still need experimental evidence support. (3) Viral infection-induced epigenetic modification—DNA tumor viruses have developed various mechanisms to affect the status of chromosome modification through the modulation of some key enzymes activities, such as DNA methyltransferase and histone deacetylase, further regulating viral and host gene expression[82]. For example, KSHV vFLIP can induce *AXL* expression potentially through *AXL* gene hypomethylation[82,83]. However, DNA methylation is considered as an important mechanism for silencing of HERVs, and hypomethylation in tumors and/or treatment with DNA-demethylating agents, such as 5-aza-2-deoxycytidine and 5-azacytidine, may lead to HERVs transactivation[84,85]. Therefore, a change in the epigenetic modification induced by viral infection may drive HERV transactivation. (4) Modification of host immune system by viral infection—previous studies have shown that the antibodies of HERVs were found in the sera of patients with autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and lupus erythematosus, indicating the association between HERVs transactivation and host immune system[6,12]. Interestingly, the envelop protein of HERVs, such as HERV-H and HERV-K family, displays immunosuppressive properties in vivo[86,87]. DNA tumor viruses have developed various mechanisms to regulate host immune system[82,88]. Thus, virus-mediated host immune system modification may cause HERV transactivation, which in turn, contributes to the development of virus-associated malignancies.

Increasingly, recent literature supports that HERVs transactivation may be a potential contributor to the development of virus-associated tumors. Thus, studies on HERVs transactivation by different tumor viruses might provide new insights and strategies for the prevention and/or treatment of these special malignancies.

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Conflict of interest
The authors declare that they have no conflict of interest.

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