KSHV Cancerogenesis and the Novel Strategies in Vaccine Design

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Abstract. Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus-8 (HHV-8) is an oncogenic virus that is associated with Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), multicentric Castleman's disease (MCD), and other immune disorders. During the millions-year-long co-evolution with human, KSHV has developed a sophisticated system to switch and balance its biphasic latent-lytic lifecycle. To date, tons of efforts have been made to unveil its lifecycle and specific cancerogenesis. However, not a single specific, effective, and widely accessible treatment for this virus has been figured out. This article reviews the basis of the KSHV lifecycle and some important factors to generate malignant cancers and then offers a few novel and feasible vaccination strategies.

Keywords: KSHV, Cancerogenesis, Cancer Vaccine.

1. Introduction

HHV8 is categorized into the gamma herpesvirus superfamily and is believed to be linked with several cancers. Firstly discovered by Yuan Chang and Patrick Moore in KS patients, in 1994 [1], this Rhadinovirus is also found to be the pathogenic agent for PEL, MCD, and in addition, some cases of plasmablastic lymphoma.

Like its sibling Epstein Barr virus (EBV), KSHV also has a distinct biphasic lifecycle comprising latent and lytic replication stages. During the latency, which usually lasts for decades, only a subset of the viral gene could be selectively expressed to facilitate the persistence of the episomal viral genome, help to escape from immunosurveillance, and intervene in the host signaling pathways. In contrast, under certain cell stress, a temporal transcriptional cascade called lytic reactivation takes place and results in the expression of the full complement of lytic genes followed by progeny virion assembly and egression. More importantly, however, both latency-associated and lytic-expressed protein and microRNA play their corresponding but also significant roles in cell transformation and tomourigenesis pathways. It is the transition between the two stages that eventually establish a lifelong persistent infection, autoimmune inflammation disorder, dissemination and metastasis of malignant cancers, and the spread from host to host.

Much effort has been paid to demonstrate the pathogenesis of KSHV and its essential key factor contributing to disease development. These investigations provide multiple therapeutic targets for novel drug designs for KSHV-associated malignancies. Herein, this article pays extensive attention to the immunotherapy of KSHV-related cancers in particular. This article will discuss some applicable options hopefully can replace the previous treatment with the problems of severe off-targeting and cytotoxicity.

2. Mechanism

2.1. KSHV lifecycle

Through technology like PCR, HHV-8 DNA can be detected in both adherent and dissociative cell lines. Specifically, they are endothelial cells, epithelial cells, and leukocytes. Numerous viral glycoproteins that help virion attachment, fusion, and entrance into the host cell are expressed by KSHV. Among them, the gH/gL complex, K8.1A antigen, and gB are thought to be the most

fundamental keys to the host cell [2]. Different glycoprotein functions differently with the host receptors, and there are still a lot more studies that are needed to elucidate the interactions. A comprehensive investigation of KSHV entry into the host can help with novel vaccination design, which may effectively block and prevent KSHV from establishing de novo infection and subsequent life-long chronic latency.

After entering into the host cell, KSHV soon unshells and decoat itself to help transfer its genome into the nucleus in an epigenetically-naive state. Then the host epigenetic modification machinery will play an essential role in converting the viral transcriptionally-permissive genome into a fully chromatinized, latent KSHV episome. During the initial establishment of latency, the early lytic gene replication and transcription activator (RTA) is expressed at a low level and it is hypothesized to be significant for the transcription of latency establishment dependent factors. As evidence shows that a transactivation cofactor of RTA can interact and activates the promoter of the major organizer of KSHV latency, the latency-associated nuclear antigen (LANA) [3]. There is one thing to mention the LANA expression could also exploit the host transcription factors instead.

LANA is dispensable in the initial establishment of KSHV latency, its axial status is mainly based on three functions. First, LANA is able to recruit host DNA or histone modification proteins to the viral genome to inhibit lytic gene expression and promote latent gene transcription. Unlike other herpesviruses, HHV-8 has a very GC-rich genomic landscape. LANA can recruit DNA methyltransferases to bring repressive epigenetic marks to the initially-unmethylated KSHV genome to broadly silence gene activity including RTA after de novo infection [4]. Second, LANA can play its role in posttranslational modification of host chromatin assembly factors in adding a small ubiquities-like modifier (SUMO) protein tag (this process is known as SUMOylation) thus relocating its cellular position or altering the protein-protein interaction surface. Third, LANA is SUMOylated itself, endowing a variety of interactions with host cellular complexes that are in charge of mitosis, DNA unwinding, and mRNA processing [5].

Once the expression of LANA is triggered by the early lytic gene such as replication and transcription activator, the entire host cell enters a generally-repressive chromatin state, followed by the formation and maintenance of latent KSHV episomal genome. During the latency, KSHV manipulates host cellular machinery via a variety of host signaling pathways, chromatin structure, and numerous expressions of viral proteins and miRNAs which are destined to strictly restrain the expression and function of RTA, and what is more important, contributed to a relatively safe escape from host immunosurveillance so that establish life-long persistent infection.

When the cellular microenvironment alters and the information is sensed and augmented by the cascade of the cellular signaling pathways, RTA activation responds quickly to stimuli in a positive-feedback mechanism, as one of the most critical targets of RTA is its own promoter. When RTA accumulates to a certain degree, it can outcompete LANA for the majority of its binding sites and overturn the latency in successfully transactivating 34 lytic genes and the subsequent complete expression of all the KSHV genes. The environmental stimuli include hypoxia, reactive oxygen species (ROS), histamine signaling, high glucose, and 1,23-dihydroxyvitamin D3, which is the biological-active form of vitamin D. The most essential signaling pathway that controls KSHV reactivation is the MAPK cascade [4].

2.2. KSHV tumorigenesis and pathogenesis

Most KSHV-seropositive tumor cells exhibit a latent gene program. Therefore, it has long been acknowledged by all that the latency, which along with life-long chronic infection serves as the hallmark of herpesvirus, plays a decisive role in KSHV-related tumorigenic properties. One major function of a latent gene like LANA and vFLIP expressed in tumor cells is to promote cell growth and to prevent latently infected cells from senescence, apoptosis, and necroptosis, thus augmenting its transformation potential. However, latency alone is insufficient to fully explain all about the KSHV-associated disease, lytic cycle is also indispensable for malignancies and disease development. Cells that go through lytic reactivation will express all the KSHV genes, in form of proteins and

microRNAs. Complete lysis kills host cells and increases the risk of immune responses, but virion synthesis and release also mean generating novel infections and replenishment of the pool of latently infected cells that might later change into transformed cells. Moreover, some lytic viral products will be secreted outside, contributing to tumor growth in a paracrine signaling manner. These are capable of inducing angiogenesis, and immunoevasion, and promote tumor development. It also permits the growth of a subset of lytic genes under some specific circumstances known as abortive lytic replication without inducing apoptosis. To date, a lot of efforts have been made to fully elucidate the lifecycle of KSHV as well as its pathogenic pathways. These great works provide a bunch of potential targets for drug design that doesn't get enough attention [7].

2.2.1 KSHV Latent factors

(1) LANA

Since LANA is so essential for the initiation and maintenance of KSHV episome in the host cells, it certainly has a lot to do with the transformation of cells. LANA can bind and inhibit the activity of p53 and Rb, collectively leading to instability of cellular chromosome, disregulation of the cell cycle, and blockage of apoptosis. LANA was also shown to be able to increase telomerase expression, which increases the lifespan or even internalizes the infected cells [4].

(2) vCyclin

The cyclin-dependent kinase cdk6 can interact with vCyclin, which is encoded by ORF72 and has constitutive and functional similarity with cellular cyclin D2. Coupled cdk6-vCyclin complex phosphorylate and promote degradation of the tumor suppressor Rb and the anti-apoptotic protein Bcl-2. That explains why there is a greater chance for patients who are infected with KSHV in their B-cell lines to develop lymphoma [6].

(3) vFLIP

vFLIP is a viral homolog of cellular FLICE (FADD-like interleukin-1 beta-converting enzyme, also called caspase-8) inhibitory protein. It can bind and prevent caspase-8 from cleavage into the active form. In this way does vFLIP contribute to be free of the host cell's self-clearance. Furthermore, vFLIP can persistently bind to IKKa and IKKb and thus upregulate nuclear factor kappa B (NFkB) signaling, implicating its transforming potential. Interestingly, the anti-autophagy action of vFLIP can prevent the vCyclin-induced senescence. From this point of view, one thing can be concluded that KSHV has developed a sophisticated regulation of both coordinated and antagonizing systems during the decades of co-evolution with human beings.

(4) The Kaposins

There are three types of Kaposins encoded by ORF K12 named Kaposin A, B, and C. It has long been identified that these viral proteins are subject to major transforming and oncogenic factors of HHV-8. Kaposin A is associated with cellular transformation and regulation of adhesion. Kaposin B stabilizes a variety of mRNAs involved in endothelial cell growth, angiogenesis, cytokine expression, and induction of inflammatory responses. However, the function of Kaposin C in the pathogenesis of PEL and other KSHV-associated diseases is still equivocal.

(5) KSHV microRNAs

Interestingly, the promoter of K12 not only facilitates transcription of the three Kaposins but also 12 viral microRNA precursors (pre-miRNAs). The host machinery Drosha and Dicer convert these 12 pre-miRNAs into 24 mature miRNAs. These viral miRNAs reside at primary latency sites in groups and they appear to regulate numerous viral and host cell's processes.

2.2.2 KSHV Lytic factors

(1) vGPCR

The terminology vGPCR is the abbreviation of viral G protein-coupled receptor, which is a seven-pass transmembrane protein that shares homology with the human IL-8 receptor. This determines that vGPCR can bind human humoral cytokines and chemokines. Additionally, this exogenous vGPCR is constitutively active even in the absence of ligand. The vGPCR activates a variety of critical cellular signaling pathways, including PI3K/Akt/mTOR, MAPK, Notch, PLD, PKC, etc. Downstream

cascade pathways activate transcription factors such as AP1, NFAT, NF-kB, HIF-1a, and CREB, which results in vGPCR-mediated expression of VEGF, VEGF receptor (VEGFR), and proinflammatory cytokines and chemokines in a paracrine secretion program. Collectively these pathways contribute to the cancerogenesis in angioproliferation, activation of chronic inflammation, and endothelial cell differentiation and transformation. Besides, studies also demonstrate the downregulation of TLR4 expression by vGPRC in endothelial cells, suggesting its another important role in immune evasion. As TLRs are pattern recognition receptors (PRR) and the trigger of antivirus cytokines such as IFNb, TNF-a, IL1-b, and IL-6. Last but not the least, there's a controversy about vGPCR's function in RTA expression and subsequent lytic reactivation, about whether it sustains, represses, or both [6] [7].

(2) K1 and K15

K1 is a single-pass transmembrane glycoprotein encoded by ORF1. Its corresponding cellular homologue is the B cell receptor (BCR). Like vGPCR, this lytic protein seems to contribute to KSHV lytic replication through the regulation of RTA expression, however, the particular process behind it still needs to be elucidated. In a similar manner to vGPCR, K1 also has a highly conserved intracellular immunoreceptor tyrosine-based activation motif (ITAM) and is also constitutively active. With the help of this kind of domain, K1 modulates the B-cell life cycle in a VEGF autocrine and paracrine loop via matrix metalloprotease 9 (MMP-9). The function in KSHV pathogenesis of K1 involves angiogenesis, invasiveness of KSHV-infected endothelial cells, increased vascular permeability, and oncogenic transforming properties as well. What's special about K1 is that studies show that K1 has the potential to interact with the HIV-1 protein Tat, suggesting its coordinative function in co-infection and cooperation with HIV-1. K15 is also a transmembrane receptor antagonizing BCR signaling, however, K15 lacks an ITAM domain and exerts its oncogenic function through cellular signaling pathways.

(3) Other lytic factors

There are many other oncogenic lytic factors produced by KSHV which have profound impacts on almost every aspect of transformation. The vIL-6, vIRFs, vBCL-2, and vCCLs are KSHV versions of its cellular homologue IL-6, IRFs, BCL-2, and CCLs respectively. They either activate or inhibit its corresponding cellular pathways and eventually lead to events including immune evasion, immortallization, and angiogenesis. RTA and ORF57 are reported to be responsible for the induction of double-strand breakages (DSBs, a kind of DNA damage), and the formation of R-loop, and consequently result in host genome instability. Moreover, K3 and K5 have an activity similar to E3 ubiquitin ligase. Their probable substrate may include human major histocompatibility complex 1 (MHC-1), indicating its role in the evasion of the innate immune response.

3. Treatment (vaccine) strategies

Though great progress has been made in the discovery of KSHV and its related disease's pathogenesis, development in the KSHV vaccine is still limited. The major reason for that might be the lack of demand and interest because the overall morbidity of KS, PEL, and other KSHV-associated malignancies remains relatively low, on the large scale around the globe. Second, since there are some treatments available for KS lesions already, even before the discovery of KSHV in 1994, it seems unnecessary to develop the KSHV vaccine. However, in some resource-limited areas like central Africa, the seropositive rate of KSHV is >50% [7]. Additionally, the approaches in being are expensive and not readily accessible in these countries. Moreover, the traditional therapies including chemotherapy, radiotherapy, and surgery face common problems: the off-target effect, cytotoxicity, limited recovery, or even the risk of worsening malignancies. So, from an epidemiology perspective, developing an affordable and effective vaccination strategy is beyond beneficial.

The biggest challenge for novel KSHV vaccine development stands within its latency nature, which explains why there is only one licensed herpesvirus vaccine till now, the Oka vaccine against varicella-zoster virus. During the thousand-year-long co-evolution with human populations,

herpesvirus has developed a complicated system to maintain its gene pool while keeping latent from the immune system. Thus, it's rather tricky to identify and eradicate the latent viral genome. One lesson that must be learned from the EBV gp350 vaccine studies is that the single-target antigen of the subunit vaccine may be insufficient to induce effective immunity. Different multiple-antigen presenting vaccination strategies should be taken into consideration.

Self-assembly virus-like particles (VLPs) are a solution for exposing a combination of antigens in a virus-like manner. Producers can express viral envelope proteins in non-human cells. After purification, these proteins can auto-assembly into empty VLPs without viral nucleic acid, yet still, keep their immunogenicity. Forming in native viral structure and morphology, VLPs can efficiently interact with dendritic cells and other antigen-presenting cells, which results in antigen-based adaptive T and B immune responses. In a recent study, scientists built a KSHV VLPs structure utilizing KSHV glycoprotein gpK8.1, gB, and gH/gL to target APC. They constructed a series of chimeric HHV-8-NDV fusion and expressed them in mammalian CHO cells. As a result, they successfully obtained KSHV VLPs that are valid to induce serum neutralizing antibodies (nabs) in mice. However, in the antibody response studies in immunized mice demonstrated that the antibodies titer induced by VLPs incorporating KSHV proteins went through a sharp decrease after about 70 days [8].

Another vaccination delivery is via mRNA. It has recently got heated as an attractively feasible candidate. Compared with viral vector-based and peptide-based vaccine, the nucleic acid vaccine has superiority in overcoming vaccine resistance as well as a broader T cell response. At the same time, the mRNA vaccine is non-infectious. And as producers can control the in vitro transcription (IVT) mRNA production to ensure its purification, there should be fewer safety concerns as the consequence. After being internalized into the cytoplasm, multiple full-length antigens can be translated and presented directly by APCs to elicit both humoral and cell-based immune responses. The most concerns about mRNA vaccine in the early attempts include mRNA instability, challenging in vivo delivery, and usual innate immunogenicity. Nonetheless, modern technological innovations have solutions. First, various modifications can be added to the backbone and untranslated regions of mRNA to make it more RNase-resistant. Also, more efficient mRNA in vivo deliveries has been achieved by utilizing vehicles including lipid nanoparticles (LNPs), high-molecular polymers like PEI, and peptides. Moreover, improved purification can allow mRNA products free of dsRNA contaminations, thus decreasing the initiation of exogenous nucleic acid-specific innate immunity. With more and more maturing IVT methods applied to the production of mRNA, mRNA vaccines have obtained supreme advantages in large-scale manufacturing and affordability. So far, a new kind of technology called self-amplifying mRNAs (SAM) has gained extensive attention. By combining viral antigen transcripts of interest with RNA-depending RNA polymerase (RdRP), SAM can achieve long-lasting immunogenicity in lower required dosages [9].

As so many KSHV-expressed genes have been unveiled to play different critical roles in pathogenesis, the targets of interest in drug design shouldn't be limited to the envelope proteins alone. Studies reported that the core latent protein LANA has the potential to initiate KSHV-specific CD8 T-cell responses. Also, some early and late lytic proteins like K12/Kaposin can also trigger corresponding immunity [10][11]. These significant regulatory proteins in controlling KSHV latent and lytic lifecycle might be the decisive factors that induce KSHV-specific immune responses, more investigations are needed to reevaluate the feasibility of developing a vaccination strategy.

According to others, the likelihood of a successful live-attenuated virus vaccine increasing immunity against a broad spectrum of viral antigens is higher. However, there is no evidence to prove its security of not establishing latent infection. Even though UV- processed KSHV has a superior antibody response. Thus, the removal of latent infection and trying to weaken viral lytic replication without losing its immunogenicity are served as the two major tasks of developing a live virus-based vaccine. Another obstacle lies in the lack of proper animal models for KS development and the evaluation of vaccine efficacy. HHV-8 can only infect primates other than humans, there is no readily accessible primate model for further studies, and this will greatly hamper KSHV vaccine development

for sure. All the KSHV vaccination strategies can only be tested by mouse infected with MHV-68, a rodent gamma-herpesvirus closely related to KSHV [12].

4. Conclusion

First identified in 1994, KSHV is not just an endemic cancerogenic virus, but a globally-spread threat to lives, especially in areas where therapies like CAR-T are unaffordable. In this situation, a novel, potent, and mass-producible vaccine that can induce long-term immunity against KSHV infection will serve as a cost-effective candidate treatment. Attempts in EBV vaccine development and the studies of rodent herpesvirus are lessons that must be learned. In the future, more investigations in digging out the potential of the mRNA vaccine may help solve the KSHV-related issue.

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