Seminars in Cancer Biology 19 (2009) 153–157

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

# Epigenetic regulation of Kaposi's sarcoma-associated herpesvirus replication

Shara N. Pantry, Peter G. Medveczky*

*Department of Molecular Medicine, College of Medicine, University of South Florida, 12901 Bruce B. Downs Boulevard, MDC7, Tampa, FL 33612, USA*

ARTICLE INFO

*Keywords:*
Kaposi's sarcoma-associated herpesvirus
Epigenetics
Histone acetylation
Chromatin structure
DNA methylation

ABSTRACT

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma and B-lymphocyte disorders, primary effusion lymphoma (PEL) and Multicentric Castleman's Disease (MCD). KSHV usually exists in a latent form in which the viral genome is circularized into an extrachromosomal episome. However, induction of lytic replication by environmental stimuli or chemical agents is important for the spread of KSHV. The switch between latency and lytic replication is regulated by epigenetic factors. Hypomethylation of the promoter of replication and transcription activator (RTA), which is essential for the lytic switch, leads to KSHV reactivation. Histone acetylation induces KSHV replication by influencing protein–protein-associations and transcription factor binding. Histone modifications also determine chromatin structure and nucleosome positioning, which are important for KSHV DNA replication during latency. The association of KSHV proteins with chromatin remodeling complexes promotes the open chromatin structure needed for transcription factor binding and DNA replication. Additionally, post-translational modification of KSHV proteins is important for the regulation of RTA activity and KSHV replication. KSHV may also cause epigenetic modification of the host genome, contributing to promoter hypermethylation of tumor suppressor genes in KSHV-associated neoplasias.



## 1. Epigenetic regulation of gene expression

In eukaryotic cells, DNA methylation at CpG dinucleotides plays an important role in the regulation of gene expression and pathogen recognition. In the human genome CpG dinucleotides are relatively rare, occurring in frequencies approximately 25% as would be expected, because over time methylated cytosine residues undergo an irreversible deamination to become thymine (reviewed in [1]). However, some regions of the human genome contain stretches of DNA greater than 0.5 kb with a C + G content of 55% or greater (reviewed in [2]). These regions, called CpG islands, make up ~1% of the human genome and are often associated with the promoters of genes. Methylation at CpG dinucleotides near or within promoters effectively diminishes gene transcription by blocking the access of transcriptional machinery to promoter regions by steric hindrance (reviewed in [1]). This transcriptional repression is important for preventing the expression of foreign DNA, such as retrotransposons and proviral sequences (reviewed in [3]). Most CpG dinucleotides in the human genome are methylated. In contrast, unmethylated CpG dinucleotides are a signature of bacteria and other pathogens, and serve as potent stimulators of the vertebrate immune system.

Eukaryotic genomic material is packaged as chromatin, which is comprised of DNA wrapped around core histone proteins (reviewed in [2]. The tails of core histones which aid in chromatin packaging are often covalently modified after transcription (reviewed in [4]. These histone tail modifications play an important role in determining chromatin structure and condensation, both of which are important in regulating transcriptional activity. For example, acetylation of lysines on core histone tails, by histone acetyltransferases (HATs), leads to unraveling of chromatin and transcriptional activation. On the other hand, counteracting deacetylation by histone deacetylases (HDACs) leads to chromatin condensation and transcriptional silencing. Modified histones also recruit numerous other proteins, including transcription factors, and chromatin remodeling complexes, which use energy liberated from cleaved ATP molecules to move nucleosome along the length of DNA, resulting in assembly or disassembly of nucleosome cores and serve as an effective method of controlling transcription (reviewed in [5]).

The process of malignant transformation includes both genetic and epigenetic changes. In human cancers, methylation and histone modification patterns are often altered; this greatly influences chromatin structure and subsequent gene expression. Since a considerable amount of human cancers are attributed to viral infections, it is important to consider epigenetic modifications in the

*Abbreviations:* 5-AzaC, 5-Azacytidine; DNMT, DNA methyltransferase; EBV, Epstein–Barr virus; HAT, histone acetyltransferase; HDAC, histone deacetylase; IE, immediate-early; KSHV, Kaposi's sarcoma-associated herpesvirus; LANA, latency associated nuclear antigen; MCD, Multicentric Castleman's Disease; PEL, primary effusion lymphoma; NaB, sodium butyrate; TR, terminal repeat; TPA, tetradecanoylphorbol acetate.

* Corresponding author. Tel.: +1 813 974 2372; fax: +1 813 396 9188.
*E-mail addresses:* spantry@health.usf.edu (S.N. Pantry), pmedvecz@health.usf.edu (P.G. Medveczky).

1044-579X/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcancer.2009.02.010

context of how it affects the function of tumor viruses. Here we will discuss the role of Kaposi's sarcoma-associated herpesvirus (KSHV) in neoplasia formation, as well as the mechanism which DNA methylation, histone acetylation, and chromatin structure affect viral gene expression. In brief, epigenetic alterations by in KSHV associated neoplasias will also be discussed.

## 2. KSHV and neoplasia

KSHV, also known as Human Herpesvirus 8, is a member of the γ2 herpesvirus family, which includes Epstein–Barr virus (EBV) and the simian Herpesvirus saimiri. Members of this family are characteristically able to induce the formation of distinct neoplasias in natural or experimental hosts (reviewed in [6]). KSHV was first discovered by its association with Kaposi's sarcoma, a common AIDS-associated neoplasia of endothelial origin [7]. Additionally, KSHV also causes two B-lymphocyte disorders, primary effusion lymphoma (PEL) and Multicentric Castlemans Disease (MCD), which are characterized by proliferation of B-cells in the body cavities and the lymph nodes, respectively [8].

## 3. KSHV replication and latency

KSHV genome structure and gene expression pattern varies depending on its replication state. Herpesviruses exist in two replication states: lytic or latent. After primary infection, KSHV actively replicates using viral machinery and new virus particles are produced and released by host cell lysis (reviewed in [9]). During this stage, the KSHV genome is linear and the entire viral genome is expressed. Lytic gene expression begins with the expression of immediate-early (IE) genes that regulate the expression of other viral genes [10]. Expression of IE genes occurs independent of viral replication, and afterwards, early and late genes are expressed. These include genes important for genome replication as well as structural proteins.

The lytic switch of KSHV, RTA (replication and transcription activator), is encoded by ORF50 of the KSHV genome [11]. During latency, ORF50/RTA expression is repressed; however, RTA may be activated by physiological conditions, such as hypoxia, or by pharmaceutical agents ([12,13], and [14]). RTA activation triggers the start of the lytic replication cascade. RTA binds to numerous cellular and viral proteins and functions by directly transactivating various KSHV promoters. In a mechanism mediated by octamer binding protein-1, RTA also binds its own promoter and exhibits auto activation [15], possibly allowing KSHV to respond to cellular stimuli. Interestingly, expression of RTA alone is enough to disrupt KSHV latency and induce the expression of lytic genes [11] and the presence of dominant-negative RTA mutants eliminates viral reactivation [16].

After initial infection, KSHV may establish lifelong latency in B-cells [8,17]. Throughout latency, viral gene expression is highly regulated and only a few viral genes are expressed. The latent KSHV genome is circularized by joining of GC rich terminal repeats (TRs) at the ends of the viral genome to form an extrachromosomal circular episome [18]. This episome is tethered to the host chromosome by the latency associated nuclear antigen (LANA), which also functions to regulate episome replication by host cell machinery [19]. LANA, a phosphoprotein expressed in latently infected cells (reviewed in [6]), is essential for the segregation of episomes to host daughter and persistent KSHV infection [19]. Additionally, LANA promotes the maintenance of latency by associating with the ORF50 promoter [20] or binding cellular factors which normally interact with ORF50. After extended periods of latency, KSHV infections may be reactivated and the lytic gene expression may restart, however, latency is the default pathway of KSHV.

A balance between latent and lytic gene expression is important for the pathogenesis of KSHV. Latent viral proteins, such as vFLIP and LANA serve to inactivate tumor suppressors and block apoptosis (reviewed in [9]). However, lytic replication is also important for transmission of the virus in the population and in the pathogenesis of KS. KSHV protein, vIL-6, which is more highly expressed during the lytic cycle, promotes cellular growth and angiogenesis, while protecting against apoptosis (reviewed in [6]). Additional evidence for the importance of lytic replication includes the fact that inhibition of active KSHV replication by gancyclovir reduces the incidence of KSHV in HIV-infected individuals (reviewed in [21]).

## 4. Regulation of KSHV latency by DNA methylation

In the majority of PEL cell lines, such as BCBL-1, BC3, and JSC1, HHV-8 is found in a latent state. Nevertheless, the lytic cycle may be induced *in vitro* by treatment with chemicals that induce epigenetic changes. More specifically, DNA methyltransferase inhibitor 5-Azacytidine (5-AzaC), HDAC inhibitor sodium butyrate (NaB) and HAT inducer, tetradecanoylphorbol acetate (TPA) are all stimulators of KSHV lytic replication ([14] and [22]). The ability to study latently infected cells in the context of reactivation by 5-AzaC, NaB, or TPA allows researchers to elucidate the mechanism by which different epigenetic modifications control the switch between KSHV latency and lytic cycle.

Deamination of methylated cytosines to form thymines occurs spontaneously, and results in a reduction in the frequency of CpGs in the human genome, or CpG suppression (reviewed in [21]). Low level of CpG suppression, which is expressed as a ratio of observed CpGs versus expected CpGs, suggests that a genome has been subject to extensive DNA methylation. As herpesviruses persist in their host for long periods of time, they are also subject to CpG methylation and may exhibit CpG suppression. Unlike other γ-herpesviruses, the KSHV genome does not exhibit global CpG suppression [14]. This suggests that KSVH is not subject to extensive methylation. However, localized CpG suppression does occur at the promoters of specific genes, such as ORF50 and LANA.

Bisulfite sequencing of latently infected BCBL-1 cell lines reveal that during latency the ORF50 promoter is highly methylated [14]. Additionally, this promoter methylation was seen in biopsy specimens from patients with MCD, KS, and PEL. In all cases, treatment with TPA resulted in demethylation of the ORF50 promoter, Furthermore, demethylation induced by 5-AzaC caused lytic reactivation and IE (ORF50), early (vIRF), and late (K8.1) gene expression. This demonstrates that control of ORF50 promoter methylation is important for the induction of lytic replication.

## 5. Histone modification and chromatin remodeling affect KSHV replication

Histone acetyltransferase inducer TPA functions to promote KSHV lytic cycle by activating transcription factors and enhancing their DNA-binding activity [23]. RTA responsive promoters, including the RTA promoter itself, often contain C/EBPα (CCAAT/enhancer-binding protein alpha) binding sites; the binding of the C/EBPα transcription factor to the ORF50 promoter is a key step in the TPA-mediated induction of KSHV lytic replication [22]. TPA stimulates the expression of the C/EBPα protein which is stabilized by KSHV RTA and RAP (replication-associated protein) and enhances its autoregulation. However, without RTA or RAP, TPA cannot induce C/EBPα transactivation [23]. Wang et al. found that the activity of the AP-1 transcription factor is also important in early activation of the RTA promoters during KSHV lytic cycle, in that AP1 DNA-binding activity was increased as early as one hour after TPA treatment [23]. This increased AP-1 activity may be a result of