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Connivance, complicity or collusion? The role of noncoding RNAs in promoting gammaherpesvirus tumorigenesis.

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Abstract

EBV and KSHV are etiologic agents of multiple types of lymphomas and carcinomas. The frequency of EBV^+ or $KSHV^+$ malignancies arising in immunocompromised individuals reflects the intricate evolutionary balance established between these viruses and their immunocompetent hosts. However, the specific mechanisms by which these pathogens drive tumorigenesis remain poorly understood. In recent years an enormous array of cellular and viral noncoding RNAs (ncRNAs) have been discovered, and host ncRNAs have been revealed as contributory factors to every single cancer hallmark cellular process. As new evidence emerges that gammaherpesvirus ncRNAs similarly alter host cell pathways, viral factors dysregulate host ncRNA expression, and novel viral ncRNAs have yet to be discovered, we examine the contribution of small, non-miRNA ncRNAs and long ncRNAs in gammaherpesvirus tumorigenesis.

1. Gammaherpesviruses, noncoding RNAs, and cancer.

It is estimated that viral infections contribute to 15–20% of all human cancers. Among these, the highly ubiquitous gammaherpesviruses Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) play a major role, with a strong association to the development of a range of malignancies. These include Burkitt's B cell lymphoma, diffuse large B cell lymphoma (DLBCL), Hodgkin's Disease, nasopharyngeal carcinoma (NPC), and gastric carcinoma (GC), which are linked to EBV infection; Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD), which are linked to KSHV infection; and post-transplant lymphoproliferative disease (PTLD), which can result from either EBV or KSHV infection [reviewed in [1]]. Although the lifelong chronic infections associated with these agents are typically asymptomatic, gammaherpesvirus infections that occur coincident with dysregulated host immune responses or environmental cofactors can lead to lymphoproliferative and neoplastic disorders. Thus the variety and incidence of gammaherpesvirus-associated tumors reflects

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both (a) the inherent oncogenic capacity of these viruses and (b) the delicate counterpoise between these viruses and their hosts.

While small RNA or DNA viruses can evade some innate immune effectors to establish an infectious foothold, most of these pathogens are ultimately cleared by the immune system. In contrast, large dsDNA gammaherpesviruses must necessarily be equipped with sufficient genetic armaments to navigate their complex lifestyles. In addition to evading the initial wave of innate immunity, gammaherpesviruses must subvert adaptive B and T cell immune responses, replicate to a sufficient level for successful host dissemination, establish lifelong latent infection, and when called upon, reactivate replication to a level sufficient for inter-host dissemination. Accordingly, these viruses must be capable of hijacking host cell machinery that in some situations requires an elaborate cascade of viral gene expression to replicate viral DNA and produce new virus particles, and in other situations requires silencing of most viral genes to establish the exquisitely restricted gene expression program that is requisite for stable maintenance of long-term latency.

To maintain the flexibility to carry out this wide range of activities, gammaherpesviruses have acquired the ability to either commandeer host cell pathways or mimic molecular strategies used by the host. Indeed, for each discovery of a new host cell pathway, gammaherpesviruses have proven again and again the ability to modulate those pathways for their advantage. Likewise, for nearly every discovery of a molecular strategy used by host cells, gammaherpesviruses have been found to have either circumvented or adopted a similar strategy. Such is the case with our emerging understanding of noncoding RNAs (ncRNAs). In the last 15 years, advances in high throughput sequencing and bioinformatics have led to a new appreciation of the abundance and variety of ncRNA species expressed in mammalian cells. Among these are (i) the class of translationally repressive 21–23 nt microRNAs (miRNAs), which are well characterized elsewhere and will not be covered here [2], (ii) a variety of small RNAs ranging in size from 25 to 300 nts which include small nucleolar RNAs (snoRNAs) and PIWI-interacting RNAs (piRNAs) [3,4], and (iii) the very large and diverse class of long noncoding RNAs (lncRNAs) that are currently defined more by their length (200 nt or larger) than by any specific common structural or functional feature [5,6]. Together there are thought to be more than 25,000 host ncRNA species [7].

Recently a plethora of new reports have revealed important roles for other ncRNAs in nearly every cellular process, and most importantly, additional genomic and mechanistic studies have begun to link dysregulation of ncRNAs to a wide range of cancers. Thus it stands to reason that gammaherpesviruses must exploit similar strategies during latent infection, likely both by encoding unique viral ncRNAs and by altering the expression or function of essential host ncRNAs. At present, the list of known EBV and KSHV ncRNAs is small. However, new genomics platforms and more in-depth analyses of herpesvirus genomes have begun to reveal the presence of numerous previously unknown noncoding transcripts. Such viral ncRNAs have become the subject of more intense scrutiny as our understanding of the potential functions of these molecules has begun to evolve, and as new technologies for examination of ncRNA function have emerged.

2. Host noncoding RNAs in cancer.

Mounting evidence has recently implicated ncRNAs as key players in central regulatory pathways such as proliferation, apoptosis and immune recognition that are frequently dysregulated in cancer [8–10]. Although a direct mechanistic link between an individual ncRNA and a specific tumorigenic event has not yet been elucidated, it would seem only a matter of time before such a finding is uncovered: numerous studies have now demonstrated altered expression of small and long ncRNAs in specific malignancies, and links between altered ncRNA expression and tumor cell growth have been recently elucidated.

2a. Host small noncoding RNAs.

Although small ncRNAs were generally considered to be subtle molecular control elements, recent unbiased screens for cancer-associated genes have led to increased interest in these molecules as potential tumor-promoting molecules. For example, 60–300 nt snoRNAs form small nucleolar ribonucleoprotein complexes that guide post-transcriptional modification and processing of other RNAs [11–13]. However, the C/D box snoRNA U50 may also hold tumor suppressor activity, as downregulation of U50 significantly correlates with some forms of prostate and breast cancers [14,15,] and the U50 locus is an important translocation breakpoint in diffuse large B cell lymphoma [16]. In contrast, increased expression of the H/ACA box SNORA42 is frequently associated with non-small cell lung cancer [17] and correlates with poor clinical prognosis [18]. Likewise, although the 25–33 nt piRNAs form complexes with PIWI proteins ostensibly to silence transposable genetic elements [19], overexpression of piRNA-823 in multiple myeloma increases global DNA methyltransferase activity and promotes angiogenesis [20]. Similarly, piRNA-651 is upregulated in some gastric, lung, breast, liver, and cervical cancers, and inhibition of piRNA-651 leads to cell cycle arrest and reduced proliferation [21].

2b. Host long noncoding RNAs.

LncRNAs are a very large and loosely defined collection of transcripts that are greaterthan 200 nt with no appreciable coding potential. hough our understanding of the mechanisms by which lncRNAs exert their influence over cellular processes is still evolving, several themes have emerged including functioning as molecular scaffolds for DNA, RNA and/or proteins as a means to alter chromatin, regulate mRNAs, regulate protein function, or alter the stoichiometry of miRNA/mRNA interactions. Moreover, lncRNAs have been shown to be involved in all cancer hallmark pathways [10], and altered expression of numerous host lncRNAs have been detected in a wide spectrum of tumor samples [22], suggesting the potential for the direct involvement of dysregulation of these molecules in tumorigenesis. Indeed, several examples to support this possibility have recently been revealed. For example, increased expression of the 2.16 kb lncRNA HOTAIR is a significant predictor of breast cancer metastases and death [23]. Similarly, the 6.5 kb lncRNA MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) was first identified as an upregulated gene in lung cancer metastases [24], and marked increases in MALAT1 expression have been subsequently observed in lung, bladder, breast, and cervical cancers, suggesting that dysregulation of MALAT1 may have significant implications for the progression of numerous types of tumors.

Thus these and numerous other host ncRNAs are now being revealed as important players in the larger picture of tumor initiation and promotion. Even as our understanding of the biochemistry and function of these types of molecules in cellular processes increases, our understanding of fascinating classes of small RNAs continue to emerge, including vault RNAs [25], Y RNAs [26], and extracellular RNAs (exRNAs) [27]. As only a very small percentage of these host molecules have thus far been examined, it seems likely that future research will elucidate many more host ncRNAs that contribute to tumorigenesis.

3. Gammaherpesvirus noncoding RNAs.

Through co-evolution with their mammalian hosts, gammaherpesviruses appear to have adapted the use of ncRNAs to their advantage, both through expression of their own unique ncRNA molecules and dysregulation of host ncRNAs. The EBV EBERs, first described in 1979, were among the first ncRNAs ever reported [28,29]. Since that time, an array of different ncRNA molecules have been revealed to be encoded by gammherpesviruses, including a large number of miRNAs, an assortment of unique small noncoding RNAs and lncRNAs. Among these, miRNAs have thus far received the most attention and are described elsewhere [30–34]. Here, we will focus on other gammaherpesvirus-encoded small and long ncRNAs. Thus far, only a small number of these molecules have been studied; however, the themes derived from those studies (**outlined in** Fig. 1), along with the new discovery of numerous novel gammaherpesvirus transcripts, illustrate the vast potential for the contribution of ncRNAs to the oncogenicity of these viruses.

3a. Gammaherpesvirus small noncoding RNAs: multifunctional RNAs expressed in all latently infected cells and tumors.

The Epstein-Barr virus encoded RNAs (EBERs).—The 167 nt EBER1 and 172 nt EBER2 are the two most abundant viral transcripts in latent EBV-infected cells [28,29]. Unlike most ncRNAs, EBER1 and EBER2 are non-polyadenylated RNAs transcribed by RNA polymerase III [28,35,36]. Despite the restricted viral gene expression programs operational during EBV latency, EBER transcripts accumulate to high levels in the nucleus of latently infected cells and tumor cells [37], suggesting that these molecules may be key players in chronic infection and tumorigenesis. As is typical for most ncRNAs, the ascribed cellular functions of the EBERs have been derived primarily from the discovery of their protein interactions, including with: the RNA binding proteins La, L22, and AU-rich element binding factor (AUF)-1 [38]; the antiviral immune sensors protein kinase R (PKR) [39], retinoic acid inducible gene- (RIG-I) [40], and toll-like receptor 3 (TLR3) [41]; and the transcription factor PAX-5 [42].

Not surprisingly, this array of molecular interactions may directly impact cellular functions that contribute to the tumorigenic potential of EBV. For example, numerous studies have demonstrated that EBV-negative BL B cell lines, NPC epithelial cell lines, and GC epithelial cell lines expressing EBERs are resistant to apoptosis, display increased proliferation, and demonstrate a tumorigenic phenotype [43–52]. Moreover, transgenic mice constitutively expressing EBER1 in the lymphoid compartment develop lymphoid hyperplasia or B cell lymphoma at a high frequency [53].

While these studies clearly demonstrate the potential for the EBV EBERs to contribute to tumor initiation and/or promotion, defining the contribution of EBERs to tumorigenesis in the context of EBV infection remains a difficult and controversial problem, in part due to the necessary reliance of the field on *in vitro* studies. For example, while two groups have reported that loss of EBER from EBV strains did not affect primary B cell transformation *in vitro* [54,55], another study demonstrated a dose-dependent reduction of EBER-deficient EBV required to transform cells and significantly slower proliferation of resulting clones [56]. These conflicting results illustrate the complications of studying individual viral genes in the context of intact viruses, which frequently have redundant mechanisms for achieving similar strategic outcomes. Moreover, none of these experiments address the function of the EBERs in *in vivo* tumorigenesis. Thus, although these latter findings demonstrate the potential of the EBERs to contribute to transformation, and underscore the molecular and cellular data indicating potential EBER functions that promote tumor cell fitness, much further work remains to determine the true function of the EBERs in tumorigenesis.

The murine gammaherpesvirus tRNA-miRNA encoded RNAs (TMERs).—Like its human virus counterparts, murine gammaherpesvirus 68 (MHV68) establishes lifelong latent infection in B cells and causes lymphoproliferative disease and B-cell lymphoma in immunocompromised hosts, making it an excellent model for the study of EBV and KSHV oncogenic factors. MHV68 encodes eight pol III-transcribed 200–250 nt ncRNAs [57–60] known as TMERs [61]. Each MER harbors a tRNA-like element (vtRNA) followed by one or two downstream pre-miRNA hairpins. Through noncanonical processing, the TMERs yield up to 8 different vtRNAs and up to 28 mature miRNAs [58,62–65]. Like the EBV EBERs, the MHV68 TMERs are highly expressed in latently infected cells and in virus-associated tumors [57,66], suggesting that these ncRNAs may play similar roles in chronic infection and oncogenesis. Consistent with this concept, targeted mutation of all eight TMERs reduces latent infection [61], particularly within the memory B cell population [61]. Notably, during long term infection, a similar TMER mutant virus displays an increased load of cells carrying viral genome [67], suggesting that the TMERs may regulate the balance of lytic and latent infection. Strikingly, TMER mutation also completely ablates MHV68-induced lethal pneumonia [61], demonstrating the substantial potential for such gammaherpesvirus ncRNAs to contribute to *in vivo* pathogenesis.

Relatively little is known about the molecular mechanisms by which the TMERs function. However, it is now apparent that the T ERs are not simply primary miRNA transcripts, but instead may carry out biologically important functions through the use of alternately processed intermediates. To varying degrees, processing of each TMER yields intermediates that retain the vtRNA but lack one or both pre-miRNA hairpins [62,63,68]. Some of these species are highly stable, supporting the concept that individual TMERs may encode multiple functional RNA elements. For example, an MHV68 mutant lacking TMER4 expression replicates normally *in vivo*, but is highly impaired for hematogenous dissemination [68,69], resulting in a substantial reduction in the establishment of peripheral latency. While an intermediate containing TMER4 vtRNA and one hairpin is sufficient to convey wild-type functionality, mutation of miRNA seed sequences within the remaining hairpin have no effect. Thus, as is the case for many ncRNAs, the secondary structure of the

TMER4 hairpin is likely a key determinant of function. The TMER vtRNA sequences themselves may also hold some biological activity, as restoration of the TMER1 vtRNA alone in a combined TMER mutant herpesvirus is sufficient for partial recovery of lethal pneumonia [70]. Thus the TMERs likely act as multifunctional ncRNA elements that directly participate at multiple stages of virus infection including dissemination and latency, and clearly contribute to the genesis of disease.

The Herpesvirus saimiri U RNAs (HSURs).—Herpesvirus saimiri (HVS) is a gammaherpesvirus that establishes asymptomatic lifelong latency in squirrel monkeys, its endemic hosts. In contrast, HVS infection leads to aggressive T cell leukemias and lymphomas in New World monkeys [71], and transforms marmoset T cells *in vitro* [72]. HVS encodes seven small ncRNAs called Herpesvirus saimiri U RNAs (HSURs), which are pol II-derived, but nonpolyadenylated. HSURs range in size from 75 to 143 nt, and like the EBERs and TMERs are abundantly expressed in latently infected cells and tumor cells [73–77].

The HSURs appear to structurally and functionally mimic a subset of cellular small nuclear RNAs (snRNAs) that regulate splicing through their association with Sm proteins in RNPs [75]. The 5’ ends of HSUR1 and 2 also interact extensively with AU-rich element (ARE) binding proteins [78,79], which normally regulate the stability of several subsets of cellular mRNAs. Interestingly, HSUR interaction with ARE binding proteins results in the upregulation of a small subset of genes that are hallmarks of T cell and NK cell activation [80].

The high degree of HSUR conservation among HVS strains implies an important role for these molecules during infection, but their specific functions during *in vivo* infection and tumorigenesis remain largely unknown. Although the HS Rs are dispensable for *in vitro* transformation of T cells [81,82], cells transformed with HVS lacking HSUR1 and 2 have a significantly slower growth phenotype [82], indicating that HSURs may also enhance the proliferative capacity of infected cells. Moreover, HSUR1 and 2 contain near perfect miRNA seed sequence matches for host miRNAs miR-16, miR-27 and miR-142–3p [83], and recent evidence indicates that HSUR2 directly recruits these miRNAs to host mRNA targets of HSUR2 binding, resulting in target mRNA repression [84]. Consistent with a potential role for the HSURs in HVS tumorigenesis, HSUR2 mRNA targets include mRNAs in retinoblastoma, p53, and apoptosis pathways, and HSUR2-mediated repression of host mRNAs confers resistance to apoptosis.

3b. Gammaherpesvirus long noncoding RNAs: regulators of chromatin and transcription.

The KSHV polyadenylated nuclear (PAN) RNA.—To date, comparatively less is known about the existence and function of viral long ncRNAs as compared to viral small ncRNAs. KSHV expresses several potential noncoding transcripts antisense to known ORFs [95–101]. Among these, the 10 kb antisense-to-latency transcript (ALT) is of great interest due it position antisense to the major KSHV latency locus. ALT was first revealed through genome-wide tiled microarray studies [96], and has since been validated and resolved through additional molecular approaches [102]. However, as is the case for most other KSHV potential lncRNAs, the function of the ALT during infection and pathogenesis remains unknown.

In contrast, the KSHV polyadenylated nuclear (PAN) RNA is the most well-studied gammaherpesvirus lncRNA to date [reviewed in [103]]. PAN RNA is a pol II-transcribed, 1.08 kb capped transcript that was initially identified in KSHV-infected Kaposi's sarcoma lesions and primary effusion lymphoma (PEL) cell lines [100,104–106]. PAN RNA is abundantly expressed throughout lytic replication, during which time it accumulates to a remarkably high level [100]. Accumulation appears to result largely from a 79 nt ENE element that acts as an intramolecular clamp for the PAN RNA poly(A) tail and prevents nuclear exonuclease activity [107,108]. Notably, this finding led to the discovery of other lncRNAs (eg, host MALAT1) which carry stabilizing ENE-like elements [109–111] and the identification of a nuclear RNA decay pathway mediated by the poly(A) binding protein PABPN1 [112]. Additional factors also appear to block PAN RNA decay, including virus-mediated nuclear localization of the cytoplasmic poly(A) binding protein PABPC1 [113,114], direct binding to the viral protein ORF57 [115,116], and interaction with the nuclear mRNA export protein ALYREF [115,117–119].

In the context of KSHV infection, PAN RNA has been implicated in several functions including as a regulator of transcription and chromatin remodeling. Knockdown of PAN RNA in KSHV-infected cells, or infection of cells using a KSHV mutant carrying a partial PAN RNA deletion, results in significantly reduced viral gene expression and subsequent virus production [113,120,121]. PAN RNA associates with numerous viral gene promoters, as well as a subset of cellular gene promoters [121–123]. Like HOTAIR and other cellular lncRNAs, PAN RNA may regulate transcription at these sites through recruitment of histone modifying enzymes [121,123]. Importantly, although these interactions clearly influence viral fitness, it is apparent that that the presence of this incredibly abundant ncRNA also impacts cellular function. Indeed, PAN RNA associates with the promoters of multiple host genes, including those encoding proteins involved in cell cycle, cell death, and immune function [121–123]. Moreover, PAN RNA expression is sufficient to induce proliferation of several different cell types[123], strongly suggesting that this KSHV lncRNA could contribute to tumorigenesis.

The EBV BamH1 rightward transcript (BART) RNAs.—The EBV BART RNAs were initially identified as highly abundant transcripts expressed in nasopharyngeal carcinoma samples and cell lines [85–87]. Subsequent work has reported BART RNA expression in all EBV-associated diseases, including highly abundant expression in gastric carcinoma tissues [88]. Although the BART RNAs harbor several open reading frames (ORFs), BART-derived proteins have not been detected from endogenous translation [89,90]. Instead, the BART RNAs appear to serve dual roles as pri-miRNA transcripts that produce up to 44 miRNAs [91,92], and as lncRNAs, at least some of which localize primarily to the nucleus [89,93,94]. It is likely that many lncRNA-associated functions of the BART RNAs have yet to be revealed. However, recent evidence indicates that at least one spliced BART isoform significantly alters epithelial cell transcription independent of miRNA formation, resulting of the reduction of expression of multiple genes, including genes whose products promote the induction of apoptosis, the unfolded protein response, and cell migration [94]. Notably, the set of host cell genes perturbed by this nuclear BART RNA overlapped with a large subset of genes that was significantly altered following EBV infection, indicating that the BART lncRNAs may play

a central role in the host cell transcriptional changes observed in EBV-associated epithelial tumors.

3c. Discovery of new gammaherpesvirus RNAs.

Our growing understanding of the functions of these known ncRNAs provides important insight into their potential contributions to gammaherpesvirus infection and disease. However, it can be argued that these examples represent only a small fraction of all gammaherpesvirus ncRNAs. As has been the case for mammalian genomes, the application of tiled microarray and next generation sequencing technologies to herpesviruses genomes has revealed unexpectedly widespread transcription [95,124–127]. Many of these regions do not contain canonical ORFs and thus may harbor previously unknown ncRNA genes. However, the high density of gammaherpesvirus genomes, with multiple overlapping transcripts and the potential for complex alternative splicing, has greatly hampered efforts to fully define the viral transcriptomes. Thus while short sequencing reads of such regions has revealed new genomic transcript features, global delineation of specific transcripts within these regions has thus far been impossible.

Fortunately, recent advances in sequencing technology and computational pipelines have facilitated the demarcation of individual overlapping transcripts within these regions. In particular, single molecule real time (SMRT) long-read sequencing has provided a critically important step forward by allowing reproducible reads lengths over 10 kb. While individual SMRT reads may reflect bona fide full - length transcript isoforms, sequencing limitations including 5’ truncations nevertheless necessitate validation of each output structure. Although this can be accomplished for individual isoforms using laborious classical molecular techniques, the use of alternative genomics platforms coupled with the computational pipeline TRIMD has enabled global bioinformatic validation of SMRT sequencing transcript structures [127].

Application of this approach to EBV-infected B cells has already yielded a plethora of new information about the EBV transcriptome [127], including more precise determination of the 5’ and 3’ ends of nearly two-thirds of previously annotated EBV transcripts, and the identification of 296 novel polyadenylated EBV transcripts. Of these, 65 are predicted to be noncoding. Likewise, the application of this approach to MHV68 has led to the first global annotation of MHV68 transcripts, including the discovery of at least 29 putative ncRNAs (REF in revision). These findings reveal the breadth of ncRNAs encoded by gammaherpesviruses and give insight into the depth to which these viruses may utilize such ncRNAs to modulate the host.

4. Dysregulation of host ncRNAs by gammaherpesviruses.

As exemplified by the ncRNAs described above, gammaherpesviruses implement a remarkable array of tactics to alter host cell functions for their benefit. To date, most functional outcomes have been measured in terms of modulation of host protein functions. However, with our recent gains in knowledge about the enormous breadth of host ncRNA molecules and functions, we should have every expectation that these gammaherpesvirus also target host ncRNAs for their advantage. Recent work with KSHV supports this concept,

and further indicates that such alterations of host ncRNAs may also contribute to tumorigenic phenotypes (Fig. 1).

LncRNA expression profiling of endothelial cells revealed that the expression of hundreds of host lncRNAs is dysregulated during KSHV infection, including 325 with increased and 533 decreased expression [128]. Despite the limited available information about the function of most lncRNAs, at least 54 of these dysregulated transcripts have been previously shown to be aberrantly expressed in human cancers. These include the well-studied lncRNA HOTTIP, which is upregulated in numerous malignancies [129], ANRIL, an oncogenic lnc NA that promotes proliferation in numerous cancers [130], UCA1, an oncogenic lncRNA that is upregulated in several tumors [131], and MEG3, a tumor suppressor lncRNA that is lost in a wide range of malignances[132].

Interestingly, altered expression of many of these lncRNAs was dependent on KSHV miRNA targeting [128], and retrospective analyses of Argonaute crosslinking immuno precipitation (CLIP) data sets from KSHV- or EBV-infected cells revealed thousands of host lncRNAs within these viral and host miRNA targetomes respectively, suggesting that miRNA/lncRNA interaction are a novel paradigm of gene expression[133]. Some lncRNAs including ANRIL were also significantly dysregulated by KSHV latency proteins, demonstrating that at least in some cases this virus utilizes multiple strategies to alter host lncRNA expression.

The true benefits of modulation of host lncRNAs for gammaherpesviruses remain to be understood. However, even from these initial studies it is clear that altered expression could impact tumorigenicity: knockdown of KSHV-upregulated UCA1 reduced both proliferation and cell migration induced by KSHV. Thus these findings demonstrate that dysregulated host lncRNA expression could have significant functional consequences for the infected cell, and strongly suggest that this is yet another common strategy by which gammaherpesviruses hijack host cell processes.

5. Concluding Remarks.

In 2005, the discovery of tens of thousands of host ncRNAs [7] forever altered our perception of the mammalian transcriptome. Since that time, it has become apparent that at least a subset of these ncRNAs play important roles in nearly every important cellular process, including all of those that are cancer hallmarks [10]. Viruses themselves have long demonstrated the ability to utilize their highly efficient toolkits to surreptitiously disseminate through hosts, enter permissive cells, and commandeer crucial host pathways for their advantage. We now know that viruses carry out these lines of attack in part through the use of their own unique ncRNAs and by altering the expression of host ncRNAs. Yet, the development of new genomics platforms and computational pipelines is revealing that we have perhaps only begun to understand the depth to which these viruses utilize ncRNAs to their advantage (**see Outstanding Questions**). We are now at the forefront of the study of ncRNA biology. As this exploding field continues to elucidate the molecular mechanism by which ncRNAs act and the critical role that host ncRNAs play in tumorigenesis, the coming

years should bring an exciting new understanding of the very likely role that these molecules play in the genesis of gammaherpesvirus-associated tumors.

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Outstanding questions

- Do gammaherpesvirus ncRNAs have a specific and essential function during chronic *in vivo* infection?
- Is gammaherpesvirus manipulation of host ncRN s a viral strategy to modulate the host cell, or an indirect effect of virus alteration of host cell transcription and epigenetics?
- Do gammaherpesvirus ncRNAs directly mimic host cell ncRNA functions through the use of similar secondary structure?
- Can discovery of gammaherpesvirus ncRNA functions be manipulated for new therapeutic interventions or to prevent infection?
- Do gammaherpesviruses directly regulate host ncRNA processing machinery for their advantage?

Highlights:

- The gammaherpesviruses EBV and KSHV are ubiquitous human pathogens that are associated with a variety of malignancies including several types of lymphomas and carcinomas.
- Host cell noncoding RNAs (ncRNAs) play central roles in all cancer hallmark cellular processes.
- Dysregulation of host cell ncRNAs is associated with the development of multiple types of malignancies.
- Gammaherpesviruses encode numerous types of noncoding RNAs, including both short and long ncRNAs.
- Gammaherpesvirus ncRNAs modulate multiple host cell functions, resulting in the induction of cell proliferation and cell migration, blocking apoptosis, and immune evasion.
- Gammaherpesvirus ncRNAs are central players in latency and pathogenesis.
- Gammaherpesvirus factors dysregulate host cell ncRNAs.
- Numerous gammaherpesvirus ncRNAs have recently been discovered.

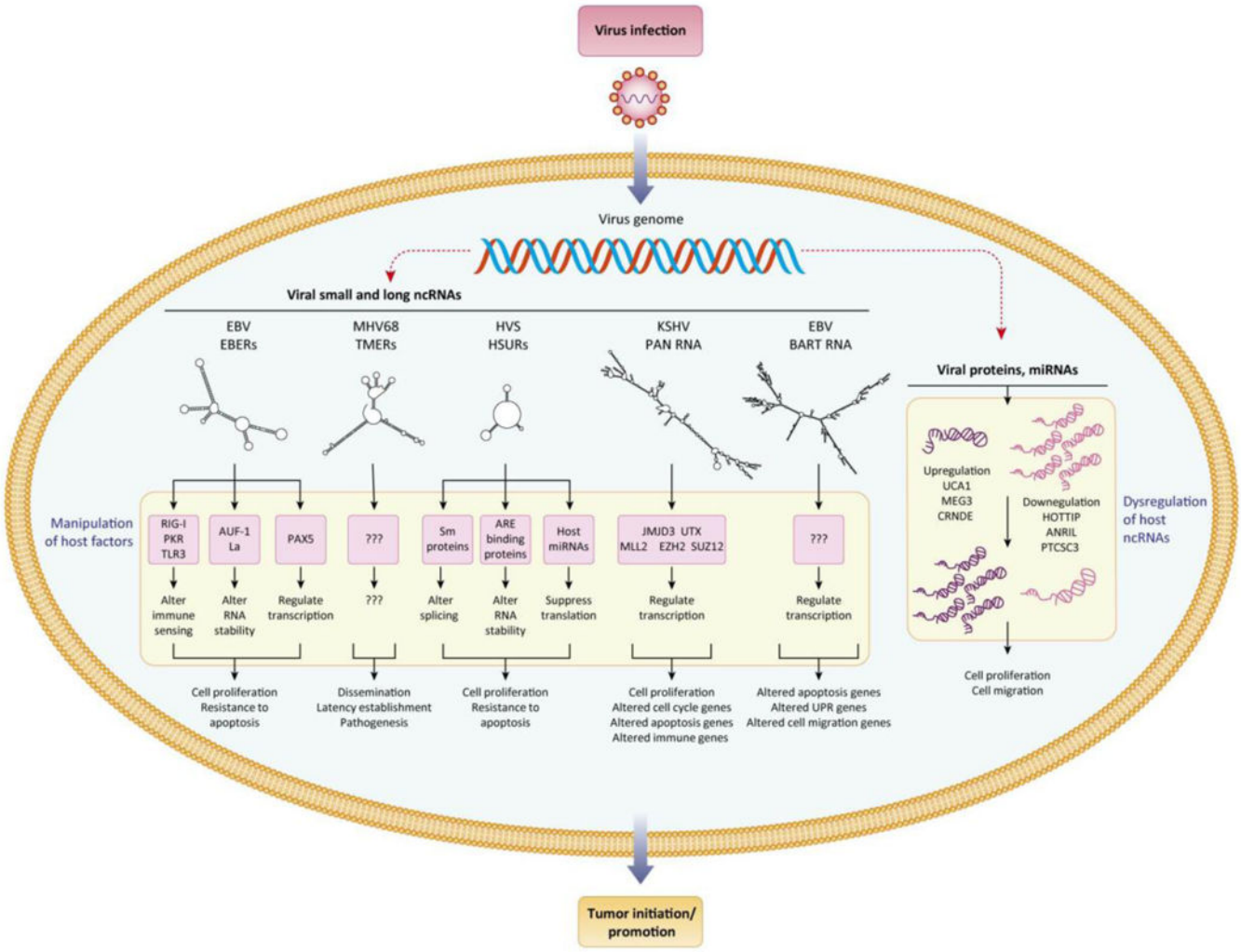

Figure 1. Virus and host ncRNAs are central players in the tumorigenic attributes of gammaherpesviruses.

Gammaherpesviruses promote infection and pathogenesis through (a) utilization of viral ncRNAs that manipulate host cell factors and (b) dysregulation of the expression and stability of host ncRNAs. Viral ncRNAs coordinate interactions with host proteins, DNA and other ncRNAs to alter immune sensing, RNA stability, RNA splicing, transcription, and translation pathways. Such host cell modifications can drive hallmark cancer processes including promoting cell proliferation, blocking apoptosis, and altering immune response networks. Viral miRNAs and proteins can suppress or enhance the expression and stability of specific host nc NAs, including numerous lncRNAs that have already been linked to cancer.