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The Role, Mechanism and Transcriptional Regulation of LAT in Herpes Simplex Virus Latency and Reactivation

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Abstract

Herpes simplex virus (HSV) infection in the human body can be latent in neurons for long time and be reactivated leading to recurrence at high rate. Currently there is no effective clinical strategy for the prevention and treatment of the disease relapse. HSV LAT gene is expressed in large quantities and lytic genes are turned off leading to HSV latency. Disruption of the gene expression is thought to cause HSV reactivation and disease relapse. To reveal the essence of HSV latency and reactivation, we summarized and innovatively classified the role, mechanism and transcriptional regulation of LAT in HSV latency and reactivation. This review may have important implications for future studies on HSV latency and reactivation, HSV disease prevention and treatment, and safer and more effective oncolytic HSVs (oHSVs).

Keywords

Herpes Simplex Virus (HSV), Oncolytic Herpes Simplex Virus (oHSV), Latency-Associated Transcript (LAT), Reactivation, Immediate-Early Gene (IE Gene)

1. Introduction

Herpes simplex virus (HSV) can be latent in trigeminal neurons in the host for

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several years or establishes a lifelong latent infection [1] [2] [3]. HSV causes a variety of human diseases [4], including infectious corneal blindness [5], gastrointestinal disorders, esophageal disorders and genital herpes infections during both primary and recurrent infections [6] [7] [8]. During primary infection, HSV-1 causes diseases that range from asymptomatic infections and cold sores, to blinding stromal keratitis and lethal encephalitis. Whereas HSV-2 infection usually leads to genital herpes [9] [10]. After a primary infection, HSV can be latent in the ganglia. Latent HSV can become reactivated causing disease relapse by evading host immunity, such as macrophages natural killer cells and interferon [11]. HSV can also spread between cells without reaching the extracellular environment. HSV is cleared by three types of immune responses in the host body: Specific cytotoxic T cells, specific delayed hypersensitivity, and antibodies. When the HSV specific antibodies reach the appropriate level, they can prevent HSV invasion of the nervous system. HSV latency makes it clinically impossible to treat HSV diseases.

It's known that latency associated transcript (LAT) is the only viral gene with high-level expression during HSV latency-reactivation cycle [12]. The LAT plays an important role in promoting latent infection by promoting cell survival, inhibiting apoptosis or other mechanisms of cell death [13] [14], repressing lytic gene expression [15] [16], and facilitating heterochromatin accumulation of the viral genome [17] [18]. It is controversial whether LAT can be successfully translated into proteins. One study showed that LAT gene did not encode any functional protein [19]. However, the LAT region contains numerous open reading frames (ORFs). Some researchers believed that LAT, or regions in the LAT gene, coded for proteins [20] [21] [22]. It was also suggested that the proteins encoded by HSV LAT in the ORFs were probably transient and difficult to detect. LAT-encoded proteins have not been experimentally detected [23]. At present, the hypothesis that LAT can encode proteins is still in the stage of experimental exploration, which needs further verification. It is of special significance to clarify the relevant mechanism of latent infection and recurrence of this virus to effectively prevent and treatment of HSV infection and recurrence [24].

In addition, tumors consist mainly of transformed cells, such as tumor stem cells [25] [26] [27] [28]. Recently, HSV has been successfully used to treat tumors as oncolytic HSV (oHSV) [29] [30] [31] [32]. oHSVs replicate preferentially in tumor cells but not in normal cells [33]. Research showed that the molecules, such as herpes virus entry mediator (HVEM) and three of its ligands (BTLA, CD160, and LIGHT), were up-regulated due to the presence of LAT in trigeminal ganglion (TG) of latently infected LTα -/- mice [34]. LTα induces apoptosis [35] while LAT has antiapoptotic function [36]. However, the mechanism of LAT against apoptosis is still not fully understood. LAT down-regulates the activation of apoptotic caspases 3, 8 and 9 [19]. *In vitro*, the cleavage of caspase 8 and 9 was inhibited in wild type HSV infected nerve 2A cells, while LAT deletion mutant viruses did not [37], indicating that the function of LAT was achieved by inhibiting the activation of pro-apoptotic caspases.

The presence of LAT is a marker of HSV latency and its expression is essential for the establishment, maintenance and reactivation of HSV latency in neurons [24] [38]. Thus, deletion of LAT can reduce latency and reactivation of HSV infection to construct safe oHSV [39]. Recent studies have shown that deletion of the LAT gene from the HSV genome can significantly improve the efficacy and safety of oHSV [40] [41].

This review highlighted the hypothesis, evidence and the mechanisms of LAT related HSV latent infection and recurrence, and the multiple functions of LAT were analyzed at the levels of transcription and translation. Our review of the latest research progress of LAT will provide new insights for the further understanding of HSV infection and therapy, and future construction of better oHSVs.

2. LAT in HSV Latency

2.1. Current Theory of LAT Mediated HSV Latency

HSV enters the cell through a fusion between the viral envelope and the cell membrane. Once inside the cytoplasm, the capsid is dissociated and transported to nuclear pores via tubule-related motional proteins, where the viral genome is released into the nucleus in a circular configuration [42]. It then begins to express immediate early (IE) genes (coding for regulatory proteins), early gene expression (especially proteins coding for DNA replication devices), and late gene expression after DNA synthesis begins [42]. It is not yet clear exactly how the HSV latent state is established and reactivated. Here we summarized several latency and reactivation mechanisms to provide new ideas for future research (**Table 1**).

Latency is a state of virus infection, in which through interaction with the host cell viruses limit their own gene expression, keep their genome into a silent state, but remain in the host neuron cell [43]. HSV particles are retrograde to the neuronal cell body, releasing viral DNA into the nucleus. When LAT is expressed the HSV genome binds to nucleosomes (specifically nucleosome histones H3K27me3 and H3K9me3/2) to form tight chromatin and silence the lytic genes, promoting HSV latency [18].

Under latent conditions, LAT and several miRNAs remain highly expressed, silencing the lytic gene expression, especially immediate early (IE) gene expression, to inhibit virus replication and promoting the survival of infected neurons [44] [45], thus effectively promoting the establishment of HSV latency [46]. Therefore, the viral genome is in a non-replicating state in sensory neurons [47]. During latency HSV is not detected at the original site of infection [48]. ICP0 protein can promote LAT transcription and silence viral lytic gene expression by histone modification and heterochromatin formation [49]. ICP0 may be a target for antiviral drugs against latent infection. HSV-1-KOS/M with basic LAT promoter or LAT gene 5' end deletion became latent in about 30% of neurons [50]. HSV-1-KOS/M with LAT deletion became latent in about 10% of neurons. It has been suggested that the 2.3 kb region of LAT is the primary latency determinant.

Table 1. HSV latency and reactivation mechanisms mediated by LAT.

Latency mechanism	Latency establishment	Reactivation	HSV strain and ref.
Chromatin modification leads to lytic gene silencing and expression of LATs and miRNA.	HSV infection at the distal axon, retrograde to neuronal nucleus.	LAT induces efficient reactivation.	HSV-1-KOS/62/17/ McKrae [44]
In sensory neurons, HSV genome remains nonreplicative and viral genes are silenced except LAT.	LAT deletion analysis indicated that LAT enhances the latency establishment.	Fever, stress, and UV irradiation or abrasion.	HSV-1-17/F [51]
LAT is able to play an indispensable role in the establishment of incubation period via reducing mRNA levels of IE gene.	LAT inhibits viral replication in nerve cells	LAT enables HSV reactivation.	HSV-1-F/KOS [46]
HSV virions are transported to the cell body within the TG to suppress effectively lytic genes.	When the virus cleavage gene is suppressed, the HSV genome enters a nucleosomal associated latent state without DNA replication in the sensory ganglia.	A stress stimulus.	HSV-1-17/KOS [48]
ICP0 promoted histones and heterochromatin modifications to silence lytic gene expression.	ICP0 promoted the establishment of latency.	ICP0 accelerates the expression of LAT and lytic genes in latent infected ganglia.	HSV-1-KOS [49]

2.2. Other Mechanisms Reported in LAT Mediated HSV Latency

At present, the understanding of the process of latent establishment and maintenance of HSV is constantly updated. There are many latent hypotheses are put forward, here we summarize the LAT related latency hypotheses into the following four categories for subsequent experimental studies.

1) Repression of ICP0 by the antisense RNA of LAT. Garber [16] *et al.* found that the gene sequence of LAT overlapped the virus immediate-early gene (IE gene) ICP0 in the antisense direction. Therefore, the LAT with high expression formed a hybrid with the mRNA of ICP0 through the antisense mechanism, preventing its modification, transport and translation, and thus blocking the generation of ICP0 protein. Since ICP0 is essential for regulating HSV genome replication, inhibition of ICP0 expression shifts the virus from productive to latent infection.

2) The anti-apoptosis effect of 1.5 kb LAT. Perng *et al.* [52], found in an animal model of rabbit, the expression of the 1.5 kb LAT only of HSV mutant strains showed the same capacity of latency and reactivation to the wide type HSV. LAT played a role in the process of HSV latency and reactivation was the initial 1.5 kb LAT of primary transcript, and the 1.5 kb LAT gene sequence had no genetic overlap with the IE gene, which suggested that LAT played a role by

other mechanisms, rather than by antisense mechanisms. Perng [36] *et al.* then showed that LAT has an anti-apoptotic effect by using *in vitro* experiments to construct plasmids expressing LAT that blocked the Fas antibody induced apoptosis.

3) Repression of ICP4 by the antisense RNA of LAT and anti-apoptosis miRNA-LAT. Chen *et al.* [53] found that the expression of LAT and ICP4 had a reverse relationship, and LAT down-regulated the expression of HSV lytic gene by inhibiting ICP4 through antisense, so as to promote the establishment and maintenance of HSV latent infection. LAT also has the function of anti-neuronal apoptosis. It encodes miRNA-LAT that mediated virus anti-apoptosis effect, thus preventing HSV from being cleared due to host apoptosis [54]. Carpenter *et al.* [55] also proved that the anti-apoptosis effect of LAT gene was conducive to the reactivation of latent virus.

4) The HSV miRNAs-ICP0/4-T cells. After entering the cytoplasm, HSV rapidly dissolves the capsid and then enters the nucleus via the nuclear pore. At this point, the virus begins to express IE genes, particularly those encoding the viral replication ICP0 and ICP4 [42]. Recently, it has been shown that miRNAs encoded by HSV-1 itself have regulatory capacities for viral genes and might control the virus latency state [56]. Two of these miRNAs were shown to reduce expression of ICP0 and ICP4 *in vitro* [57], leading to viral DNA replication suppression and HSV latency. In addition, the viral miRNAs targeted viral antigen (for example ICP4) expression reduction may cause HSV immune escape from infiltrating T cells, leading to HSV latency by a noncytolytic mechanism [58].

The understanding of HSV latency mechanism has been in dispute. Although many hypotheses have been put forward, none of them can fully explain HSV latency mechanism. Perhaps HSV latency is the result of multiple mechanisms and needs to be analyzed further.

3. LAT in HSV Reactivation

3.1. Current Theory of LAT Mediated HSV Reactivation

In natural HSV human infection, factors such as ultraviolet radiation, emotional stress, fever, tissue damage and immunosuppression can induce HSV reactivation. Experiments have showed that under certain conditions, such as fever, stress, and UV irradiation or abrasion, HSV-1 strain 17/F is reactivated [51]. Many inducing factors are related to the process of stress, so scholars have been looking for the link between stress and reactivation. Some believe that LAT has no direct role in virus reactivation [14], however, others suggest that LAT plays an irreplaceable role in activation [59]. Using LAT targeted ribozyme delivered to a HSV-1 infected eye of a rabbit, one study demonstrated that HSV-1 LAT promoted effective HSV reactivation from latency [9].

LAT may promote HSV activation by inhibiting apoptosis [19]. To some extent, the survival of neuronal cells can be achieved [60] [61]. One research found that the anti-apoptotic activity of LAT is sufficient to reactivate HSV, since re-

placing LAT with any of the three tested anti-apoptotic genes reactivated HSV to wild type levels [62]. Furthermore, LAT may regulate reactivation by affecting the immune response [8]. Liu *et al.* [63] found that interdicting $CD8^+$ T cell function through monoclonal antibody could increase the *in vitro* reactivation rate of HSV in mouse ganglion, indicating that LAT may promote HSV reactivation by controlling infiltrating $CD8^+$ T cells in latent infected ganglia. How LAT regulates the $CD8^+$ T cell infiltration remains unclear. Mice infected with LAT-deficient HSV-2 [64] showed decrease of spontaneous reactivation rate, which was not related to the increase of $CD8^+$ T cells [65]. With similar latent viral load, the reactivation rate of LAT-deficient HSV-2 was lower than that of LAT restored HSV-2 [50]. Therefore, LAT may promote HSV reactivation through anti-apoptosis, $CD8^+$ T cells, or other mechanisms.

3.2. Other Mechanisms Reported in LAT Mediated HSV Reactivation

HSV LAT may be reactivated under certain stimulus conditions. Here we summarize two other reactivation mechanisms.

1) LAT mRNA was translated to a protein that promotes reactivation of latent HSV. Under the reactivation condition (**Table 1**), the viral protein produced by HSV-LAT was significantly reduced, which induced the survival of neurons and produced an efficient reactivation of HSV-1 strain KOS/62/17/McKrae [44]. However, Thomas [21] *et al.* found that a 30 kDa protein encoded by an open reading frame of 2 kb LAT exerted functions like ICP0 and promoted the growth of HSV. Their further study showed that the protein has a biological function and the regulation of the protein expression has important role in the latent infection of HSV [22].

2) The LATs may act at the initial stages of reactivation. One mechanism in neurons is to inhibit the reverse activation of the HSV-1 IE gene, which prevents the virus from successfully replicating in infected neurons with low replication rates [66] [67]. This hypothesis postulates that LATs are the only RNA expressed during the incubation period [68] [69] [70]，which may allow the viral replication cycle to bypass this inhibition during the initial phase of activation. Meanwhile, LAT can also promote the expression of the IE genes at certain conditions, allowing reactivation of HSV-1 strain F/KOS [66]. Activation conditions under stress can lead to large amounts of transcription of the lytic genes, which can activate virus recurrence [48]. ICP0 has been shown to promote virus reactivation in HSV-1 strain KOS [71].

4. Transcriptional Regulation of LAT

The high level of LAT expression in host neurons enables HSV to effectively establish latency and reactivation. To understand how LAT may regulate the latency and reactivation of HSV, here we classified the research progresses of LAT transcriptional regulation into four molecular pathways (**Figure 1**).

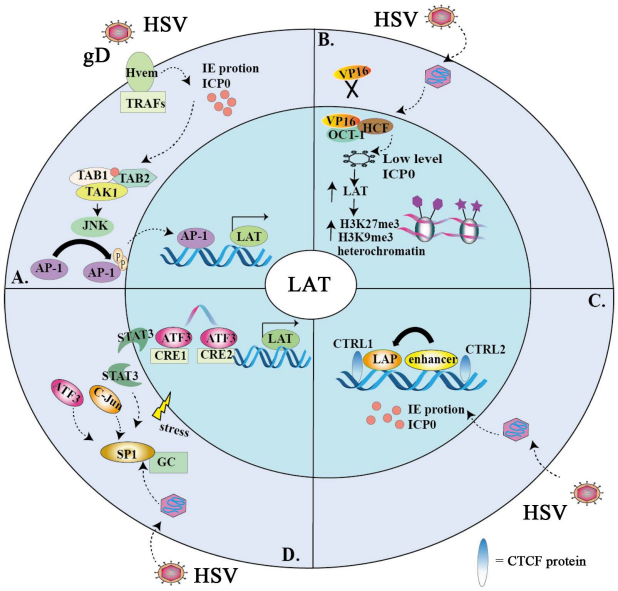

Figure 1. Four transcriptional regulation pathways of HSV LAT.

1) The ICP0-JNK-AP1-LAT pathway. HSV's gD envelope glycoprotein and its matching cell receptor, the HSV entry mediator (HVEM), a member of the TNFR superfamily whose cytoplasm area in combination with TRAFs [30]. The entry of HSV results in the synthesis of IE viral proteins involving ICP0. In turn, ICP0 catalyzes the K63-linked polyubiquitin chains with the help of Ubc13ev1A, which recognizes and interacts with the TAK1 [72]. Activated TAK1 then phosphorylates MKK6, leading to the activation of JNK kinase pathway [72]. JNK phosphorylated transcription factor AP-1. Phosphorylated AP-1 translocated into the nucleus to bind to the LAT transcription regulatory sequence, promotes transcription of LAT [49].

2) The VP16-LowICP0-LAT pathway. Multi-protein complex of VP16, Oct-1 and HCF to initiate IE transcription. However, as a capsule protein, VP16 cannot be successfully transported to ganglion nuclei with viral genomes because of certain physical losses. In absence of VP16, multi-protein complex (VP16, Oct-1 and HCF) cannot be formed, thus reducing IE gene transcription [73] [74]. Therefore, the IE gene encoding the ICP0 protein would be reduced. Low levels of ICP0 can promote both LAT and lytic gene expression in latently infected ganglia as well as total histone and heterochromatin loading on the latent HSV genome [49]. In contrast, in latently infected neuron nuclei the low levels of ICP0 expression can promote the expression of LAT and the accumulation of H3K27me3/H3K9me3 heterochromatin to silence lytic gene expression [18] [75].

3) The CTRL-CTCF-LAT pathway. When HSV enters the cytoplasm and the capsid breaks away, the viral genome begins to express IE genes. The IE genes encode the protein ICP0. It is important to note that the ICP0 encoding region is flanked by two CTCF binding motifs, the CTRL1 and CTRL2 [1]. Binding of CTRL sites, the insulators or enhancers, by the CTCF protein activates LAT

promoter (LAP) transcription, making the virus latent. Recent works defining the CTCF-insulator's role in lytic gene transcription repression of β and γ herpes viruses further support the possibility that CTCF-insulators also regulate α herpes virus latency through lytic gene repression [1].

4) The Sp1-Stat3-ATF3-CRE-LAT pathway. Sp1 protein is endogenously abundant in SK-N-SH cells [76], can interact with a variety of GC rich promoter sequences, and can recruit STAT3, ATF3, and c-Jun complexes [76]. Normally, STAT3 is present in the cytoplasm of neurons, but in a certain state of stress, STAT3 quickly migrates to the nucleus [77], functions together with ATF3, repressing neuronal cell death [78]. It has been agreed that ATF3, as a regulator of stress response, is an immediate-early response gene that plays a key role in determining the fate of cells [79]. ATF3 can combine a cAMP response element (CRE) with the typical sequence of 5'-TGACGTCA-3' in the form of homodimers or heterodimers with other members of the ATF/CREB family [80]. HSV-1 DNA contains a total of eight CRE sites, but only two are in the promoter range, particularly in promoter 1 of LAT (LAP1) [81]. ATF3 binds to both CRE sites in LAP1, forms a homodimer, and fosters increased accumulation of LAT transcripts [80] [82], and plays a critical role in the maintenance of HSV-1 latency.

5. Conclusion

HSV causes a number of diseases that are incurable due to HSV latency and reactivation and so far, no effective vaccine has been developed. Notably, researchers are focused on understanding the mechanisms of HSV latency and reactivation, where LAT played an important role. We reviewed and innovatively classified the role, mechanisms and transcriptional regulation of LAT in HSV latency and reactivation. We hope this review will have important implications for future studies on HSV latency and reactivation, HSV disease control and engineering of safer and more effective oncolytic HSVs.

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Conflicts of Interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

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