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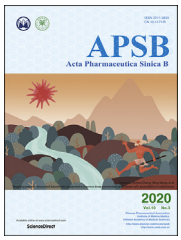

REVIEW

Disturbed Yin−Yang balance: stress increases the susceptibility to primary and recurrent infections of herpes simplex virus type 1

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Abbreviations: 4E-BP, eIF4E-binding protein; AD, Alzheimer's disease; AKT, protein kinase B; AMPK, AMP-dependent kinase; BCL-2, B-cell lymphoma 2; cGAS, cyclic GMP-AMP synthase; CNS, central nervous system; CoREST, REST corepressor 1; CORT, corticosterone; CPE, cytopathic effect; CTCF, CCCTC-binding factor; CTL, cytotoxic T lymphocyte; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; DEX, dexamethasone; GREs, GR response elements; GRs, glucocorticoid receptors; H3K9, histone H3 on lysines 9; HCF-1, host cell factor 1; HDACs, histone deacetylases; HPA axis, hypothalamo−pituitary−adrenal axis; HPK, herpetic simplex keratitis; HPT axis, hypothalamic−pituitary−thyroid axis; HSV-1, herpes simplex virus type 1; ICP, infected cell polypeptide; IRF3, interferon regulatory factor 3; KLF15, Krüppel-like transcription factor 15; LAT, latency-associated transcripts; LRF, Luman/CREB3 recruitment factor; LSD1, lysine-specific demethylase 1; MAVS, mitochondrial antiviral-signaling protein; MOI, multiplicity of infection; mTOR, mammalian target of rapamycin; ND10, nuclear domains 10; NGF, nerve growth factor; NK cells, natural killer cells; OCT-1, octamer binding protein 1; ORFs, open reading frames; PAMPs, pathogen-associated molecular patterns; PDK1, pyruvate dehydrogenase lipoamide kinase isozyme 1; PI3K, phosphoinositide 3-kinases; PML, promyelocytic leukemia protein; PNS, peripheral nervous system; PRC1, protein regulator of cytokinesis 1; PRRs, pattern-recognition receptors; PTMs, post-translational modifications; RANKL, receptor activator of NF-κB ligands; REST, RE1-silencing transcription factor; ROS, reactive oxygen species; SGKs, serum and glucocorticoid-regulated protein kinases; SIRT1, sirtuin 1; sncRNAs, small non-coding RNAs; T_3, thyroid hormone; TCM, traditional Chinese medicine; TG, trigeminal ganglia; TK, thymidine kinase; TRIM14, tripartite motif-containing 14; TRKA, tropomyosin receptor kinase A; T_{RM}, tissue resident memory T cells.

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KEY WORDS

Herpes simplex virus type 1;
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Stress

Abstract Herpes simplex virus type 1 (HSV-1), a neurotropic herpes virus, is able to establish a lifelong latent infection in the human host. Following primary replication in mucosal epithelial cells, the virus can enter sensory neurons innervating peripheral tissues *via* nerve termini. The viral genome is then transported to the nucleus where it can be maintained without producing infectious progeny, and thus latency is established in the cell. Yin−Yang balance is an essential concept in traditional Chinese medicine (TCM) theory. Yin represents stable and inhibitory factors, and Yang represents the active and aggressive factors. When the organism is exposed to stress, especially psychological stress caused by emotional stimulation, the Yin−Yang balance is disturbed and the virus can re-engage in productive replication, resulting in recurrent diseases. Therefore, a better understanding of the stress-induced susceptibility to HSV-1 primary infection and reactivation is needed and will provide helpful insights into the effective control and treatment of HSV-1. Here we reviewed the recent advances in the studies of HSV-1 susceptibility, latency and reactivation. We included mechanisms involved in primary infection and the regulation of latency and described how stress-induced changes increase the susceptibility to primary and recurrent infections.

1. Introduction

Herpes simplex virus type 1 (HSV-1), a ubiquitous human pathogen, is a neurotropic virus with a linear double-stranded DNA genome that contains more than 80 open reading frames (ORFs) and is about 152 kilo base-pairs (kbp) long. Upon primary infection, the virus replicates within epithelial cells and undergoes its typical lytic life cycle with a cascade of immediate early (IE), early (E), and late (L) genes. Then, it enters axon terminals of sensory neurons and is retrogradely transported to the corresponding sensory ganglia, usually trigeminal ganglia (TG), where a latent infection can be established. In response to a variety of stressors, the latent virus can be reactivated periodically to resume productive replication followed by the formation of infectious virus, which is anterogradely transported back to peripheral tissues or infects further neuronal cells to remain in host[1,2]. During anterograde transport, both enveloped capsids and non-enveloped capsids are detected[3]. Primary infection, latency and reactivation complete the life cycle of the overall HSV-1 infection (Fig. 1). It is also noteworthy that HSV-1 gene expression during latency and reactivation might differ between rodent and mammal models[4].

HSV-1 is commonly acquired during early childhood, primarily through oral secretions; while sexual transmission is an increasingly common cause of infection in some countries[5]. Worldwide, the global prevalence of HSV-1 is approximately 90%, and in some rural areas, the seroprevalence is even higher, up to 100%[6−11]. The success of HSV-1 infection can be attributed to its ability to establish lifelong persistent infection of the host, termed latency. In latent state, HSV-1 maintains the episomal viral genome in neuronal nuclei without producing infectious progeny for long period of time. Eventually, the virus can re-enter the lytic replication program for productive replication, a process known as reactivation. Reactivation ensures long-term persistence and dissemination of the virus to further host cells or new hosts. Furthermore, the lifespan of the latent infected cell is extended, and thus the virus is able to escape immune surveillance.

HSV-1 has raised concerns because it can cause many diseases of various severity. Acute HSV-1 infection can cause herpes labialis (cold sores)[12], gingivostomatitis[13] and eczema herpeticum[14]. It should be noted that, currently, there is no treatment to completely remove HSV-1 once an individual is infected. Reactivation of latently infected HSV-1 can cause recrudescent lesions and is the main reason for herpes viral encephalitis[15−17] and herpetic keratitis[18]. Recurrent ocular infection can lead to irreversible corneal scarring and blindness[19]. Increasing number of studies[20,21] have confirmed HSV-1 as pathogen directly related to nervous system degenerative diseases like Alzheimer's disease (AD). Reactivation of HSV-1 will increase the risk of developing AD[22]. Importantly, an amplified concentration of HSV-1 antibody and the use of antivirals can antagonize the nerve damage of AD, which has also proven that HSV-1 in latent status leads to long term damage to central nervous system[23,24]. It might likewise be the cause of Meniere's disease, an inner ear disease with spinning sensation, loss of hearing, and pressured feelings in the ear[25]. Hence, all these findings have emphasized the importance on the study of HSV-1 latent infection.

As a rapidly developing systematical medical science, traditional Chinese medicine (TCM) is a systematical medicinal science with an application history of over 2000 years in China, widely spread in Asia. At present, a broad range of research in the field of TCM is proceeding. As a treasured resource accumulated by the continuous practice of Chinese people, it has inspired many new discoveries in drug and therapeutic developments[26−29]. One of the basic theories in the TCM field is the theory of Yin−Yang balance, which is also applied as a philosophical term. Therein, Yin represents the repressive and inhibitory factors, while Yang stands for the active and aggressive factors. The confrontation, homeostasis, and transformation between Yin and Yang compose the Yin−Yang balance. In different contexts and situations, the components of Yin and Yang refer to different elements. For instance, in "Shang-Huo" syndrome, the hyperactivity of Yang, in this case is "Huo" (fire), causes increased neuroendocrine activation, hence breaking the Yin−Yang balance[30,31]. In TCM theory, the disturbance of Yin−Yang balance is an essential cause of diseases.

Stress has been defined as a non-specific reaction of an organism, which fails to respond appropriately to abnormal environmental stimuli or emotional/physical threats. Disturbing the

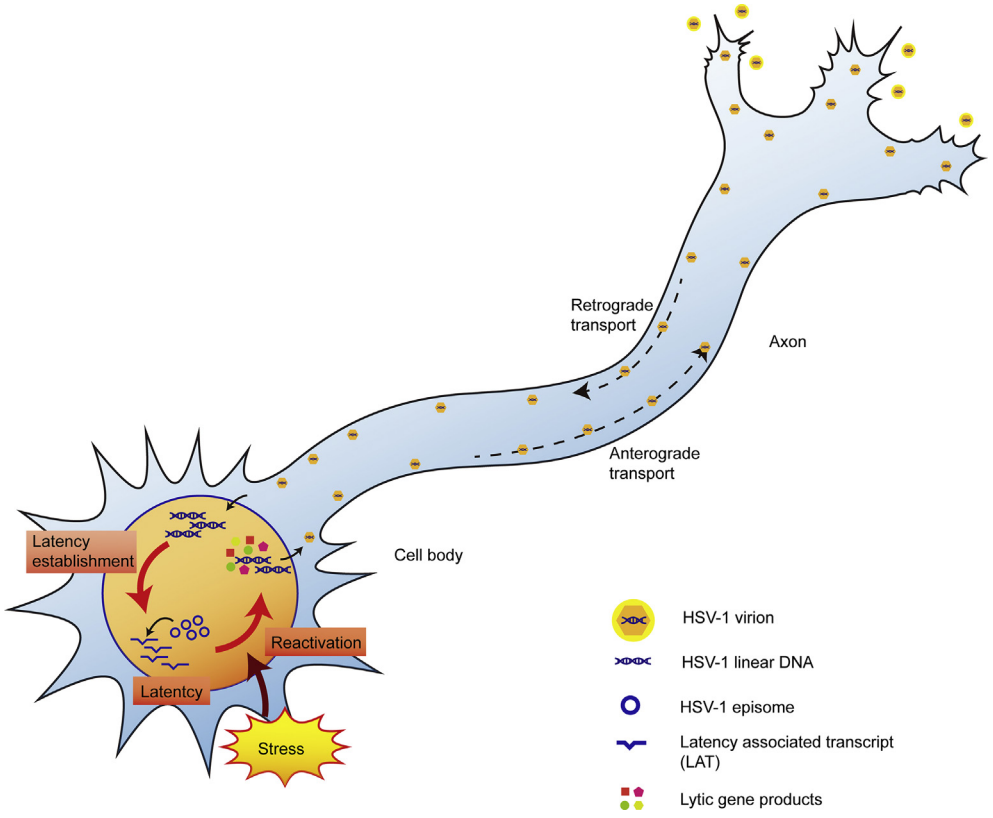

Figure 1 The life cycle of HSV-1 in neurons. During the establishment of latency, the virus invades the axonal termini through virion fusion and travels to the nucleus through retrograde transport. Then the virus enters its latency. During latency, the virus maintains itself in the neuron as an episome while silencing most of its genome and transcribing only a series of mRNAs, especially LAT. When stimulated by stress factors, the virus reactivates and starts to massively replicate its lytic gene. The proliferated virus then travels to the axon termini through anterograde transport. The complete assembly of the virion is finished in the process of egression. After the egression, the virus re-infects epithelial cells and causes recurrent lesion.

Yin−Yang balance, stress can increase the susceptibility to infectious agents, influence the severity of infectious disease and reactivate latent herpesviruses by significantly modulating the central nervous (CNS), endocrine and immune systems. TCM theory suggests that internal injury caused by excess of seven emotions, named "Qi-Qing Nei-Shang", also known as emotional stress in modern medical science, disturbs Yin−Yang balance, "Qi-Xue" and viscera balance, inducing the stagnation of Qi[32]. Chronically, the stagnation transforms into "Shang-Huo" syndrome, where Yang dominates Yin[31]. One of the typical symptoms of "Shang-Huo" is heat sore on the face, which is related to the reactivation of latent HSV-1 infection by modern medicine. Here we review the latest insights into the mechanism of stress-induced susceptibility to HSV-1 and its reactivation from latency in order to shed light to future researches on HSV-1 latency and the possible solutions to the effective control of latent HSV-1 infection reactivation.

2. Stress increases the susceptibility of HSV-1 infection by disturbing host inner Yin−Yang balance

It has been widely accepted that stress during HSV-1 infections can influence the susceptibility, infection severity and infection types[33−36]. The host defense against HSV-1 consists of three main parts: innate immunity, adaptive immunity and intrinsic immunity[37]. Therein, innate and adaptive immunity are the main defense strategies for most mitotic cells and have been investigated more thoroughly[38]. Under normal condition, the host innate and adaptive immunities perform in a cooperative and mutually restrictive Yin−Yang balance, ensuring adequate level of defense against pathogens. However, when the balance is disturbed by stimulations, the immunity balance begins to wander, which consequently leads to immune compromise.

Our previous studies[39−41] have already demonstrated that restraint stress is able to induce immune compromise, thereby increasing the susceptibility to viral infection and the severity of the infection, such as influenza; and that anti-stress treatments can reverse such changes. Under normal conditions, the morbidity of mice inoculated with H1N1 virus was about 30%, while the mice pretreated with restraint stress for 22 h showed a morbidity of 100%[42]. Survival curves, lung index, virus nucleoprotein level and immunohistochemistry results showed significantly increased disease severity[42−46]. Further investigation showed decreased mitochondrial antiviral-signaling protein (MAVS) level, natural killer (NK) cell activity and T cell activity in stressed mice, indicating significant impairment of both innate and adaptive immunity[42,45,46]. TCM formulas, such as KangBingDu Oral Liqid, are able to reverse the stress effects and reduce host susceptibility to virus, furtherly proving the feasibility to apply TCM in viral infection[42,44]. Chronic psychological stress is able to inhibit innate

and adaptive immune response towards HSV-1 including NK cell activity, HSV-specific $CD8^+$ T cell number and activity, immunity related cytokine level and lymphocyte infiltration[35]. Restraint stress prolongs the length of HSV-1 infection and increases the number of infected neurons, resulting in longer viral progeny and more severe recurrent lesions[47]. Based on the unpublished data we obtained, using corticosterone (CORT) stress model in normal and glucocorticoid receptor (*GR*) knocked-down SH-SY5Y cells, as well as restraint-stressed mice models, we have confirmed that stress hormone CORT can enhance HSV-1 susceptibility, and that such increase is largely dependent on GR. Our results further demonstrated that the GR-dependent effect of CORT on HSV-1 susceptibility is related to the inhibition of interferon regulatory factor 3 (IRF3) phosphorylation and the decrease of IFN-β, indicating an inhibitory effect of GR on innate immunity (Fig. 2). Interestingly, it has been found that, different from chronic psychological stress and restraint stress, social disruption stress can enhance the innate immune responsed to a primary HSV-1 infection in both cornea and TG of mice by increased expression of anti-viral cytokines and infiltration of macrophages, ultimately reducing the severity and frequency of future recurrences[48]. Under HSV-1 infection, tripartite motif-containing 14 (TRIM14) is likely to cleave the ubiquitin chain of cyclic GMP-AMP synthase (cGAS) and prevent it from being degraded through autophagy, ultimately enhancing IFN signaling, thus improving immune responses[49].

Besides innate and adaptive immunity, another currently under researched immunity, intrinsic immunity is closely related to the defense against HSV-1 invasion. To some extent, intrinsic immunity might play a more important role in neurons than in other cells[50]. Evidence also shown that neuronal cells are less responsive to IFN signaling than other types of cells[51]. As far as we know, there are three components for host intrinsic defense against HSV-1: autophagy, HSV-1 repressive complex and nuclear domains 10 (ND10) nuclear bodies[50]. As the virus enter the neuron, virions are degraded through xenophagy activated by pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs) and pattern-recognition receptors

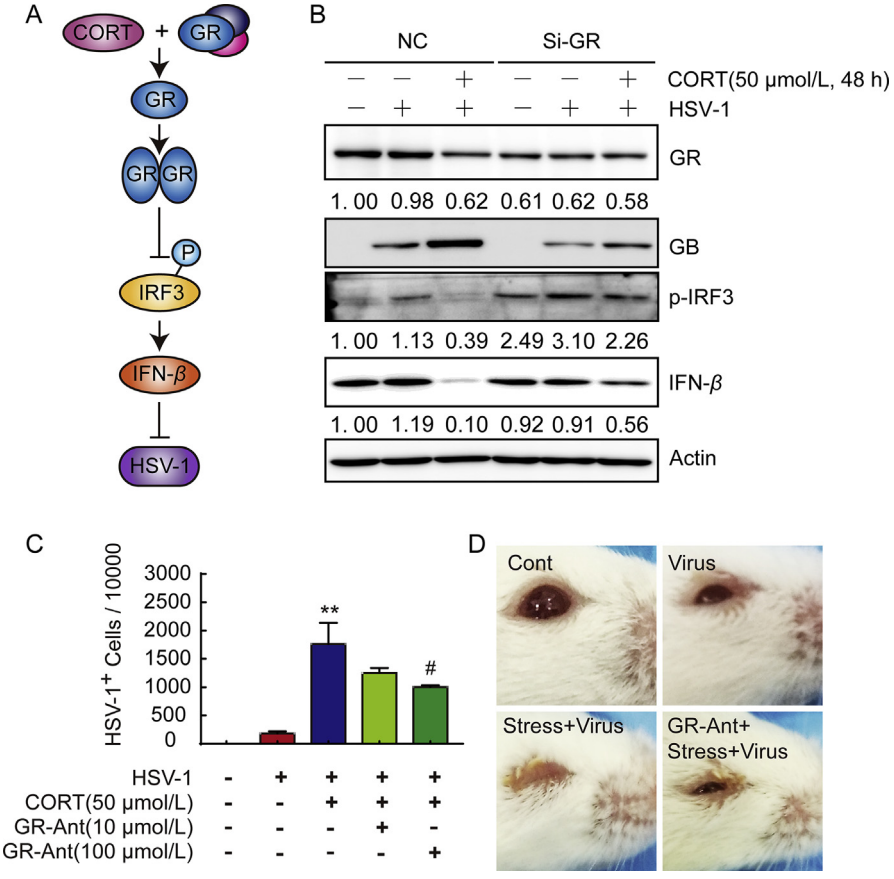

Figure 2 CORT enhances HSV-1 susceptibility, inhibits innate immunity and *GR* knock-down attenuates the effect. (A) The schematic illustration of the effect of CORT on HSV-1 susceptibility. (B) SH-SY5Y cells were transfected with vectors (NC groups) or GR siRNA (Si-GR groups). One day after the transfection, the cells were pretreated with CORT for 48 h and then inoculated with HSV-1 F strain (MOI = 1) for 24 h. In the NC groups, CORT induced significant increase in viral protein GB and decrease in IFN-β and phosphorylated IRF3, while in the Si-GR groups, the effect of CORT was attenuated. These results indicate that stress hormone CORT is able to enhance HSV-1 susceptibility. GR is indispensable for its effect, and such effect is related to innate immunity. (C) Flow cytometry results show that pretreatment of CORT significantly increased the susceptibility of SH-SY5Y to HSV-1. High dose of GR antagonist RU486 significantly attenuated the effect of CORT. GR-Ant, GR antagonist RU486. Significances were marked as $^{**}P < 0.01$ *vs*. virus group and $^{\#}P < 0.05$ *vs*. CORT+virus group. (D) Mice loaded with restraint stress showed more serious HSK phenotype than the unstressed group. RU486 alleviated the symptom. GR-Ant, GR antagonist RU486. Data shown in this figure are unpublished data of our group.

(PRRs). During virus replication, viral DNA and proteins are recognized and degraded through autophagy[51,52]. Autophagy may also be induced by IFN-β during HSV-1 replication[53]. The increased cGAS mentioned earlier is reported to interact with beclin-1, contributing to the autophagy of viral DNA[54]. HSV-1 has evolved a confrontational mechanism to counter host autophagic defense through a viral protein, infected cell polypeptide 34.5 (ICP34.5)[55−60]. It is well understood that autophagy is enhanced under stress[61,62]. On the one hand, enhanced autophagy may improve the intrinsic defense against HSV-1[57]; on the other hand, however, the increased autophagy may also prolong host cell survival and provide a more advantageous environment for HSV-1 replication[63]. Besides, whether stress-induced autophagy has the same virus clearance effect as xenophagy, a selective autophagy, remains unknown. Moreover, stress-induced autophagy upregulation might increase the degradation of cGAS, casing a loss of IFN signaling[49]. Therefore, the exact fate of HSV-1 susceptibility under stress-induced autophagy enhancement requires further investigation. The conflict between the facts that stress increases HSV-1 susceptibility and that stress enhances autophagy suggests more complicated mechanisms for stress-induced susceptibility. One possible explanation is that stress-induced autophagy increases the degradation of intrinsic defense components, such as promyelocytic leukemia protein (PML) in ND10 nuclear bodies, and hence defecting the intrinsic immune response, which is especially essential for the defense against HSV-1 infection[64]. Therefore, the stress-induced autophagy of intrinsic immune components may be a possible research direction in the future.

3. The Yin−Yang balance between HSV-1 and host cell defense: the establishment and maintenance of latency

HSV-1 is characterized by establishing latency as a non-integrated, nucleosome-associated episome in neuronal nuclei. In the process of latency establishment, new sets of Yin factors and Yang factors counteract, transform, and ultimately reach a new Yin−Yang balance between the virus and the host. When the new homeostatic Yin−Yang balance is created, the virus enters its latent state in which it resides relatively silently in the nucleus of the infected cells without producing infectious viral progeny. It is hypothesized that neuronal latency is the result of a failure to initiate the lytic cascade, which might be determined by the distinctive architecture of neurons. Therefore, here we introduce the molecular process of normal HSV-1 lytic infection process and illustrate how latency is established. The initiation of IE genes, specifically, *ICP0*, is the essential trigger for the following expression of E and L genes, indispensable for HSV-1 lytic infection[65]. Therefore, triggering IE gene expression would be crucial for entering lytic infection. With the cooperation of lysine-specific demethylase 1 (LSD1), the viral protein VP16 recruit octamer binding protein 1 (OCT1) and host cell factor 1 (HCF1) in order to form enough OCT-1/HCF-1/VP16 triplets. The triplets then bind to IE gene promoters and activate IE gene transcription[66]. ICP0 can then replace the histone deacetylases (HDACs) in the HDAC/RE1-silencing transcription factor (REST)/REST corepressor 1 (CoREST)/LSD1 repressor complex, after which the previously suppressed E and L gene expression can be triggered[67]. Only with enough VP16 entering the nucleus can HSV-1 successfully enter its lytic phase. However, specific characteristics of neurons make it especially suitable for HSV-1 to establish latency. The other two ingredients in the triplet tend to distribute differently in neurons, compared with other cells. HCF1 is more abundant in the cytoplasm than in the nucleus in unstressed neurons, while at the same time, OCT-1 is downregulated in neurons; both lead to less VP16 in the nucleus[68]. The HSV-1 genome is also closely associated with ND10 nuclear bodies, which consists of main components like PML, death-domain associated protein (Daxx) and SP100 nuclear antigen[69]. Under the stimulation of IFNs, they are able to bind closely to HSV-1 genome and inhibit its lytic replication[70]. However, such defense is also countered by viral protein ICP0, which possesses the activity of E3 ubiquitin ligase[64].

In fact, the number of viral particles that infect axons is another factor to determine whether the virus enter latency or not. The viral genome was silenced below a threshold multiplicity of infection (MOI) of 0.1; while high MOI infection resulted in productive infections[71]. To sum up, the special characteristics of neurons which are able to prevent the initiation of the viral lytic cascade and reduce the number of virions from the axons are the two important factors for the establishment of neuronal latency.

In contrast, immune surveillance seems to be dispensable for the establishment of viral latency, though the system is also critical for the early control of viral distribution as well as elimination of the replication of virus. Mice that lack the innate and adaptive immune system are still able to establish HSV-1 latency in TG[72]. Furthermore, HSV-1 latency can be established in the absence of neuronal IFN signaling[73]. Evidence in rabbit model showed that latency-associated transcripts (LATs) are able to enhance latency establishment[74], which indicates that LAT participate in the establishment of the latent state. However, since latency is still able to establish in the absence of it, and that it also accumulates in productively infected cells post-infection, LAT may not be crucial for latency establishment[75].

Whether HSV-1 can establish non-neuronal latency is unclear. However, there are several publications on cornea latent infection, suggesting that the cornea might be a possible site of latency[76]. There were evidences indicating the potential of HSV-1 latent infection in the human cornea early in the 90s[77,78]. Further studies have shown that HSV-1 DNA can be present in human corneas even though ocular herpetic disease has not been found in the corneas from animal models including mice[79−82] and nonhuman primates[83]. These results are consistent with the findings in rabbits, *i.e.*, the retrograde migration of HSV-1 DNA occurs from the transplanted corneas of rabbits latently infected with LAT positive HSV-1 to the uninfected rabbits following transcorneal epinephrine iontophoresis[75]. In addition, LAT was found in the human cornea[80], but no other transcriptional products (RNA) or expression products (protein) were observed. Given the lack of such observations, HSV-1 latency in the cornea is still disputed, *i.e.*, if the virus is truly latent in the cornea or only at a transition point along the exit path from a sensory ganglion. So far, there are three hypotheses for the presence of this DNA in human corneas: (i) operational latency; (ii) a low persistent infection in the cornea and (iii) reactivation from neuronal sites. Hence, operational latency in the cornea is very likely to be dependent on novel detection methods, which is more sensitive than present virus detection methods[84].

Maintenance of viral latency is dependent on the delicate Yin−Yang balance maintained between HSV-1 and the synergistic effects of several host factors: immunity, HSV-1 microRNAs and other factors (Fig. 3). In this context, Yang is the virus-stimulating factors, including HSV-1 virus itself, promoted glucocorticoid level, oxidational damages, etc.; Yin is the virus-inhibiting factors, such as immunity, thyroid hormone level, etc. The currently most

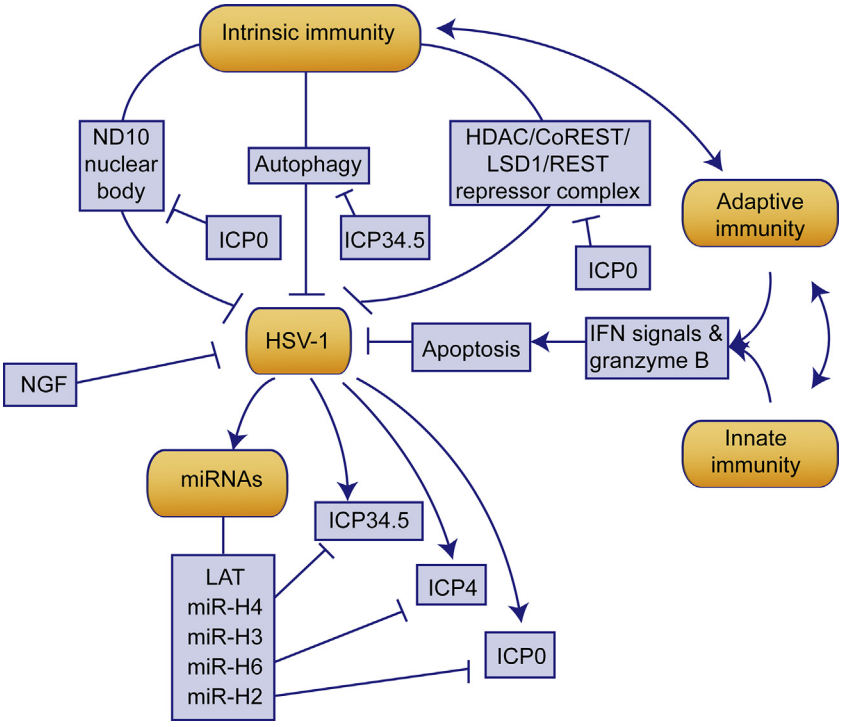

Figure 3 The interaction between HSV-1 and host cell defense during latency. During latency, HSV-1 activity is inhibited by multiple factors. Intrinsic, innate and adaptive immunity supervise HSV-1 replication while modulating each other. Intrinsic immunity inhibits HSV-1 activity mainly through ND10 nuclear body, autophagy and HDAC/CoREST/LSD1/REST repressor complex, and HSV-1 acts against them through the effect of ICP0 and ICP34.5. Adaptive and innate immunity inhibit the viral activity through granzyme B and IFN-γ secretion, leading to host cell apoptosis. Excretion of NGF by neurons inhibits HSV-1 activity. During latency, HSV-1 only expresses LAT-derived miRNAs, which are able to inhibit lytic gene expressions.

frequently used antiviral treatments for HSV-1 are DNA polymerase inhibitors including acyclovir, famciclovir, and valaciclovir[85,86]. Attenuated mutations of HSV-1 lower the virulence by deletion of *ICP0*[87], deletion of *ICP34.5*[88], expression of *ICP34.5* complementary miRNAs[89], etc. They both act as interventions of the Yang, which attenuate the Yang factors in the Yin−Yang balance and facilitate the maintenance of latency. When the organism experiences stress stimulation, the balance will be interrupted, ultimately leading to reactivation.

During latency, viral gene expression is largely suppressed except for the abundant expression of LATs and other non-coding RNAs. LAT and its associated miRNA species have been found to influence viral maintenance by suppressing HSV-1 gene expression *in vivo* and *in vitro*[90−92]. In murine ganglia latently infected by LAT-mutant HSV-1, both the neurovirulence and reactivation frequency are significantly increased[93] and the latency is impaired[94], providing further clues for the role of LAT in latency maintenance.

In addition to repressing virus-encoded gene expression, LAT can promote neuronal cell survival by inhibiting apoptosis[95], which may contribute to maintaining the latency and increasing the survival of the virus in the host. LAT's anti-apoptotic function is mediated through multiple ways. On one hand, cells are protected against cold-shock-induced apoptosis mainly by preventing the dephosphorylation of protein kinase B (AKT), a serine/threonine protein kinase promoting cell survival[96]. On the other hand, LAT expressing plasmids are able to inhibit the two major apoptosis pathways in mammals induced by caspase-8, caspase-9, and caspase-3[97−99]. LAT can prevent infected neurons from being killed by $CD8^+$ T cells through anti-apoptosis activity[98].

A set of miRNAs derived from LAT are able to target viral transcripts and inhibit HSV-1 gene expression[100]. HSV-1-miR-H6 can target *ICP4* mRNA, inhibiting the expression of the transcription factor crucial to E and L gene transcription, blocking virus lytic infection and decreasing IL-6 expression, hence promoting HSV-1 latency[101,102]. HSV-1-miR-H2, which has been associated with the regulation of latency, binds to viral *ICP0* mRNA and inhibits its expression. ICP0 plays a major role in both primary and recurrent HSV-1 infections. Its expression triggers the entry of HSV-1 into the replication cycle[93,103]. HSV1-miR-H4 inhibits the expression of the viral *ICP34.5* gene, an important lytic neurovirulence factor indispensable for promoting viral spread from TG cells to non-neuronal cells[89,104,105]. Two small non-coding RNAs (sncRNAs) derived from LAT also contribute to the decrease of lytic gene expression and apoptosis inhibition[91,106].

Another strategy employed by HSV-1 is to activate cellular glucose synthesis and glycolysis, in order to increase available energy for virus survival[107]. The activation of AMP-dependent kinase (AMPK) and sirtuin 1 (SIRT1) pathways is effective in inhibiting productive infection and protecting cells from virus related damage[108]. The modulation of AMPK/SIRT1 axis is modulated over time to suit the HSV-1 infection process[109]. An over-expression of Luman/CREB3 recruitment factor (LRF), acts as a repressor in a direct or indirect manner to inhibit essential genes of HSV-1 lytic infection[110].

Furthermore, different cytokines have different abilities to support latency and suppress lytic HSV-1 replication, providing a fundamental-level control based on neuron−virus interaction[111]. IFN-β can achieve control of the infection by enhancing the restriction of HSV-1 replication in neuronal cells[104]. IFN-β regulates LAT expression, which has a positive effect on neuron survival[73]. Nerve growth factor (NGF)-responsive receptors and signal transduction pathways are necessary to maintain latency and prevent reactivation. This is consistent with the ability of HSV-1 to establish latency in primary sympathetic neurons and Nd-PC12 cells cultured in the presence of NGF[112,113]. NGF ablation can induce HSV-1 reactivation in sensory and sympathetic neurons *in vitro* or after anti-NGF treatment *in vivo*[114]. Moreover, neurons infected with latent HSV-1 experience reactivation in the presence of antibodies to NGF[115]. The ability of NGF to maintain latency has also been proven when herpetic keratitis was topically treated with NGF[116]. It turns out that the activation of pyruvate dehydrogenase lipoamide kinase isozyme 1 (PDK1) caused by the reaction of NGF with tropomyosin receptor kinase A (TRKA) is the dominant pathway of NGF to suppress reactivation. Suppression of phosphoinositide 3-kinases (PI3Ks) induces HSV-1 reactivation and activation of AKT, the key component for PI3K pathway, which is particularly critical for the maintenance of latency[111,117]. This effect of NGF is closely related to mammalian target of rapamycin (mTOR), an important factor in the PI3K/AKT pathway, which controls the population of mRNAs that are actively translated into proteins. These proteins suppress the lytic cycle by sustaining the repressive chromatin state of the viral genome through activating eIF4E-binding protein (4E-BP)[118].

During latency, the viral genome associates with nucleosomes by chromatin remodeling. Histone post-translational modifications (PTMs) representative of euchromatin and heterochromatin are found on HSV-1 genes during latency. As a result, the regulation of latent gene expression exists at the level of epigenetic modification. Two activating euchromatin-like modifications that commonly define areas of euchromatin are acetylation of histone H3 on lysines 9 and 14 (H3K9, K14) and dimethylation of histone H3 on lysine 4 (H3K4). Indeed, during lytic infection, acetylation of H3K9 and H3K14 are enriched upon lytic gene promoters; while repressive heterochromatin-like modifications are under-represented[119]. In contrast, during latency, the actively transcribed LAT locus become enriched in acetylated H3K9 and H3K14 at the LAT promoter and enhancer, while these modified histones are absent at the *ICP0* promoter or DNA polymerase gene[120]. Meanwhile, viral lytic genes are enriched with repressive heterochromatin-like modifications such as methylated H3K27 and methylated H3K9. These observations correspond with latency feature that the LAT is abundant whilst lytic genes are silent. Furthermore, while HDAC inhibitors induce reactivation in latently infected mice[121], inhibitors of LSD1 activities that can specifically block demethylation of the repressive markers, such as H3K9me3, H3K9me2 and H3K27me3, will enhance the epigenetic suppression of the viral genome and reduce the reactivation in cultured neurons[122]. In order to prevent the spread of heterochromatin into areas of euchromatin, there must be barriers in place to keep these domains separate. CCCTC-binding factor (CTCF), a transcriptional repressor, can bind to the sites in HSV-1 genome as boundary elements[123]. Thus, the transcriptionally active LAT promoter regions will be segregated from the repressed regions of the genome and the LAT enhancer will be prevented from acting on the surrounding lytic genes[124]. These findings have suggested that epigenetic regulation may control the switch between latency and reactivation.

Systemically, HSV-1 latent infection is surveyed by the immune system through the cooperation of tissue cells and immune cells including $CD4^+$ and $CD8^+$ T lymphocytes. These HSV-1 specific T cells belong to tissue resident memory T (T_{RM}) cells. They have a longer lifespan than normal T cells, and establish a long-term residence in TG[125]. During latency, the lytic genome of HSV-1 is not completely silenced. Instead, limited lytic gene expression is frequently recognized by MHC class I molecules expressing cells, $CD4^+$ and $CD8^+$ T cells[126]. For example, local expression of viral proteins such as ICP6 and VP16 is recognized by TG-resident $CD4^+$ and $CD8^+$ T cells[127]. These facts have indicated that HSV-1 latency maintenance involves active identification and response for viral gene by host immune system. In TG, both HSV-1 specific and non-specific $CD8^+$ T cells exist and cooperate with each other, contributing to the control of latency[128]. When HSV-1 lytic gene expresses at a relatively low level, non-specific $CD8^+$ T cells can inhibit the reactivation through inhibiting ICP0 by IFN-γ. A novel autophagic response has been discovered, which is related to the IFN signaling in TG[53]. For neurons that are refractory to IFN-γ, the HSV-1 specific $CD8^+$ T cells can excrete granzyme B to degrade ICP4, and then block viral gene expression[129].

The research on HSV-1-specific $CD8^+$ T cells is particularly comprehensive. These cells have been shown important for latency maintenance[130], and the reactivation can be reduced by broadening the repertoire of HSV-1 specific T cell that is resident in TG[131]. The receptor activator of NF-κB ligands (RANKL) has the control over the induction of TG-resident $CD8^+$ T cells. The activation of RANKL also prevents cell apoptosis[132]. The balance between the survey of TG-resident HSV-1 specific $CD8^+$ T cells and their exhaustion monitored by HSV-1 LAT gene is important for the maintenance of latent status[133]. Though $CD4^+$ T cells do not have direct effect on latency maintenance, they can perform an indirect function to prevent partial $CD8^+$ T cell exhaustion[134]. Recently, it has been discovered that $CD8\alpha^+$ dendritic cells (DCs) play a more crucial role in latency maintenance than $CD8^+$ T cells[135]. Moreover, $CD8\alpha^+$ DCs are influential in T cell exhaustion, which contributes to latency maintenance[136].

4. Stress disrupts the Yin−Yang balance and causes reactivation

When the Yin−Yang balance maintained in latency is disrupted by stress, HSV-1 enters its reactivation cycle and causes recurrent diseases (Fig. 4). Stress induces the reactivation of latent HSV-1 through multiple mechanisms. When the stress is removed, the latency will be re-established due to the re-silenced viral genome[137]. Through investigations on previous researches, we summarized the molecular mechanisms in HSV-1 latency establishment, maintenance and reactivation in Fig. 5. Getting through the barriers of host immunity, HSV-1 virions enter the cell and some of them are degraded *via* xenophagy. During latency, the lytic genes are inhibited by chromosomal modifications, LAT-derived miRNAs, intrinsic, adaptive and innate immunity. Under psychological stress or hyperthermia, the increased glucocorticoids trigger reactivation by either directly activating the virus through GR activation or indirectly affecting host immunity. Under UV stress or physical trauma, ROS level increases, causing

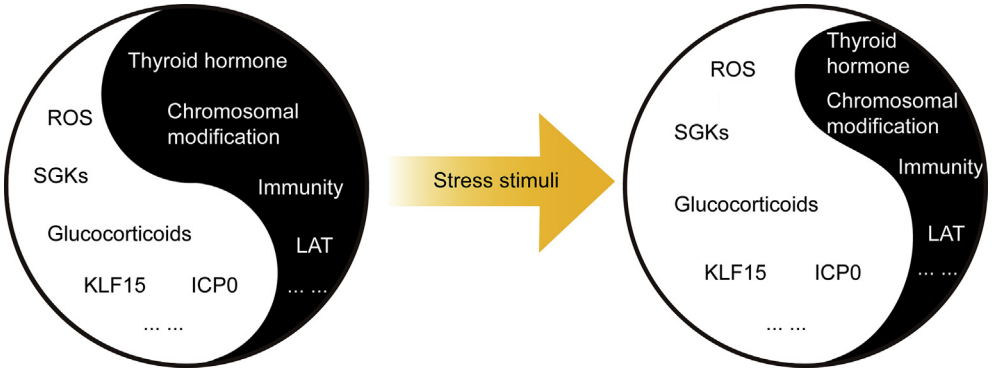

Figure 4 Stress disturbs the Yin−Yang balance between HSV-1 stimulating and inhibiting factors. During HSV-1 latent infection, the HSV-1 stimulating and inhibiting factors form a delicate Yin−Yang balance. Yin factors include virus inhibiting factors such as thyroid hormone, chromosomal modification, host immunity and LAT; Yang factors are the virus stimulating factors like ROS, glucocorticoids, SGKs, KLF15, and ICP0. When the host experiences stress stimulation, for instance, psychological stress, the Yin factors are inhibited and the Yang factors are promoted. Hence the balance is disturbed, which ultimately leads to recurrent lesions.

oxidational damage, hence inducing the reactivation. As we previously described in our publications, we have developed a successful disease susceptibility model, restraint-stressed mice model, to simulate the effects of stress on virus infection[39−46,138,139]. Hence, restraint-stress mice model can be a feasible model for stress-induced HSV-1 reactivation, as illustrated in Fig. 6.

4.1. *Stress induces hormonal changes*

It is well established that stress-induced changes of hormones such as glucocorticoids and thyroid hormone can stimulate HSV-1[140,141] and drive reactivation from latency (Fig. 7). Stress, *e.g.*, psychological stress and hyperthermia, can activate the hypothalamo−pituitary−adrenal (HPA) axis[142] and increase the concentration of glucocorticoids in the blood[143]. Glucocorticoids induce HSV-1 reactivation mainly through two distinct processes: directly affecting HSV-1 virus and indirectly promoting reactivation. Glucocorticoids activate GRs and directly activate HSV-1 transcription by interacting with the GR response elements (GREs) on virus genome. A recent study showed that when cells are treated with synthetic corticosteroid dexamethasone (DEX), GR and Krüppel-like transcription factor 15 (KLF15) cooperate to stimulate reactivation through the transactivation of ICP0[144]. Serum and glucocorticoid-regulated protein kinases (SGKs) has been reported to increase after stress stimulation and participate in HSV-1 reactivation from latency[145]. The increased glucocorticoid level also affects both intrinsic and adaptive immunities. TG-resident HSV-1-specific $CD8^+$ T cells are reduced and those survived $CD8^+$ T cells compromise functionally, which induces HSV-1 reactivation for compromising $CD8^+$ T cell surveillance. The impairment of $CD8^+$ T cells may occur *via* affecting GRs on DCs that are responsible for priming these HSV-1 specific T cells[36,146]. Interestingly, however, psychological stress might not diminish $CD8^+$ T cells *via* impairing DCs. Instead, it could be due to the decrease of T cell stimulative cytokines such as IFN-γ and IL-2[149]. Further effects of glucocorticoid upregulation by stress are significantly reduced concentrations of granzyme B and IFN-γ[142,146,147]. Also, stress has been proven to cause mitochondrial damage and to decrease MAVS expression[45]. It can decrease NK cell activity in individuals that are latently infected with HSV-1, which might be due to the reduction of IFN-γ and IL-2[148].

Thyroid hormone (T_3) is able to cause strong suppression of HSV-1 replication[149]. T_3 can activate LAT and consequently repress ICP0 expression[150]. It also has repressive effect on the HSV-1 thymidine kinase (*TK*) gene, which is important for viral reactivation[149]. Psychological stress inhibits the hypothalamic−pituitary−thyroid (HPT) axis and leads to the reduction in T_3 secretion. Hence, with the decrease of T_3 and the reduction in its suppression effects, there is an increase of ICP0 expression. It has been shown that the overexpression of thyroid receptor β1 can enhance LAT transcription and recruit H3K9me3 and H3K9me2 to repress the *TK* gene, leading to an increased virus suppression efficiency. In addition, TG neurons overexpressed with thyroid receptor β1 were less susceptive to the reactivation induced by T_3 decrease[151]. However, it should be noted that the suppression effect of T_3 only works on neuronal cells, and differential condensation of chromosome may be important in this process[152]. T_3 also regulates the expression of dynein and modify neuronal outgrowth, suggesting the specific role T_3 plays in viral transport and anti-apoptosis[153].

4.2. *Stress reverses chromosomal modification*

Under stress, the chromosomal modifications on HSV-1 lytic DNA might be reversed and viral DNA expression might be modified, which lead to induction of lytic replication[107]. This hypothesis has been supported by a number of studies, which have found that chromatin remodeling around the LAT region and surrounding lytic genes is likely to occurs after a stress stimulus[154,155]. Loss of CTCF proteins from chromosomal insulators through stress-induced phosphorylation increased the accessibility of viral genes for transcriptional activation. Replacement of demethylation of H3K27 on lytic genes with euchromatin that is triggered by displacing protein regulator of cytokinesis 1 (PRC1) complexes has been reported to have the similar function[124]. As a result, the lytic genes would productively express and eventually reactivate.

In fact, a number of transient chromatin modification have been discussed. It has been suggested that histone H3 at serine 10 undergoes a methyl/phospho switch during the first phase of

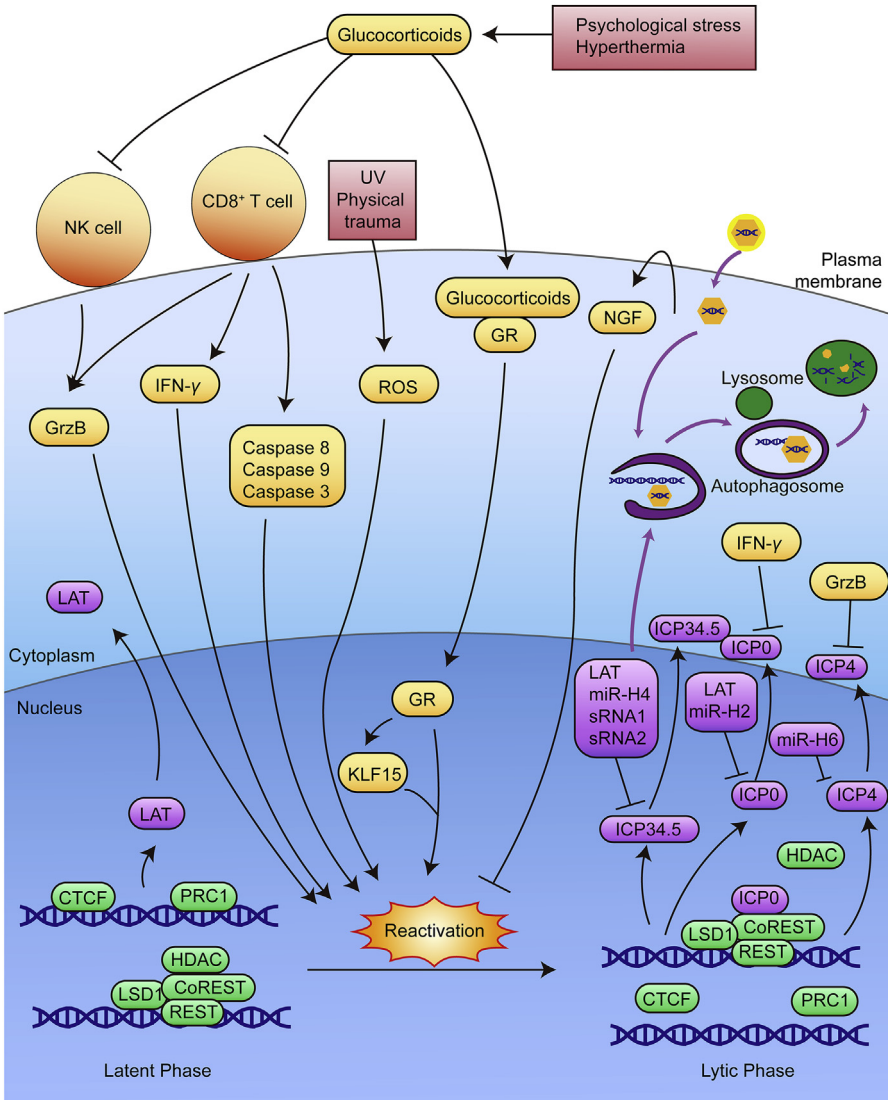

Figure 5 Molecular mechanism in an HSV-1-infected neuron during latency establishment, maintenance and reactivation. When HSV-1 virus enters the cell, some virions are degraded through xenophagy. HSV-1 tends to enter latency in unstressed neurons. During latency, HSV-1 DNA remains in its latent phase, CTCFs are attached to the insulators, and the major transcription product of IR_L gene is LAT. LAT-derived miRNAs are able to inhibit lytic gene expression. PRC1 complexes are also attached to the genome, suggesting possible inhibitory effect. The HDAC/CoREST/LSD1/REST repressor complex is in its default state to suppress the expression of E and L genes. $CD8^+$ T cells release IFN-γ, granzyme B and caspases into the neuron, and NK cells release granzyme B. IFN-γ inhibits the expression of ICP0, granzyme B inhibits the expression of ICP4, and miRNAs are able to inhibit the expressions of ICP0, ICP4 and ICP34.5. Neuron itself induces NGF which also inhibits reactivation. Psychological stress and hyperthermia increase the level of glucocorticoids, inhibiting the activity of immune cells. Glucocorticoids are also able to bind to glucocorticoid receptors and activate HSV-1 by inducing and cooperating with KLF15, thereby activating ICP0 transcription. UV and physical trauma increase ROS level. All these stress factors wake up the HSV-1 genome and lead to reactivation. During reactivation, PRC1 complexes are replaced, all CTCFs are evicted from the insulators. Consequently, the lytic genes are able to transcribe and the transcripts of IR_L gene yield ICP0 and ICP4. ICP0 then removes HDAC from the HDAC/CoREST/LSD1/REST repressor complex, stimulating the expression of all lytic genes, leading to the complete reactivation of viral genome. Some of the newly synthesized viral DNA and protein components are degraded through autophagy.

reactivation, and activation of lytic genes is achieved without the removal of H3K9me3[156]. It is then followed by VP16 synthesis in the second phase[157]. When the VP16 promotor is activated, in the absence of other lytic viral gene expressions, the expression of VP16 leads to the exit of latency and the entry of lytic cycle[158]. Therefore, modification of VP16 is required for reactivation of HSV-1 in neuronal cells[159]. Administration of an inhibitor for helicase-primase is able to suppress such reactivation, which suggests the chromatin modification mentioned above to be essential[160].

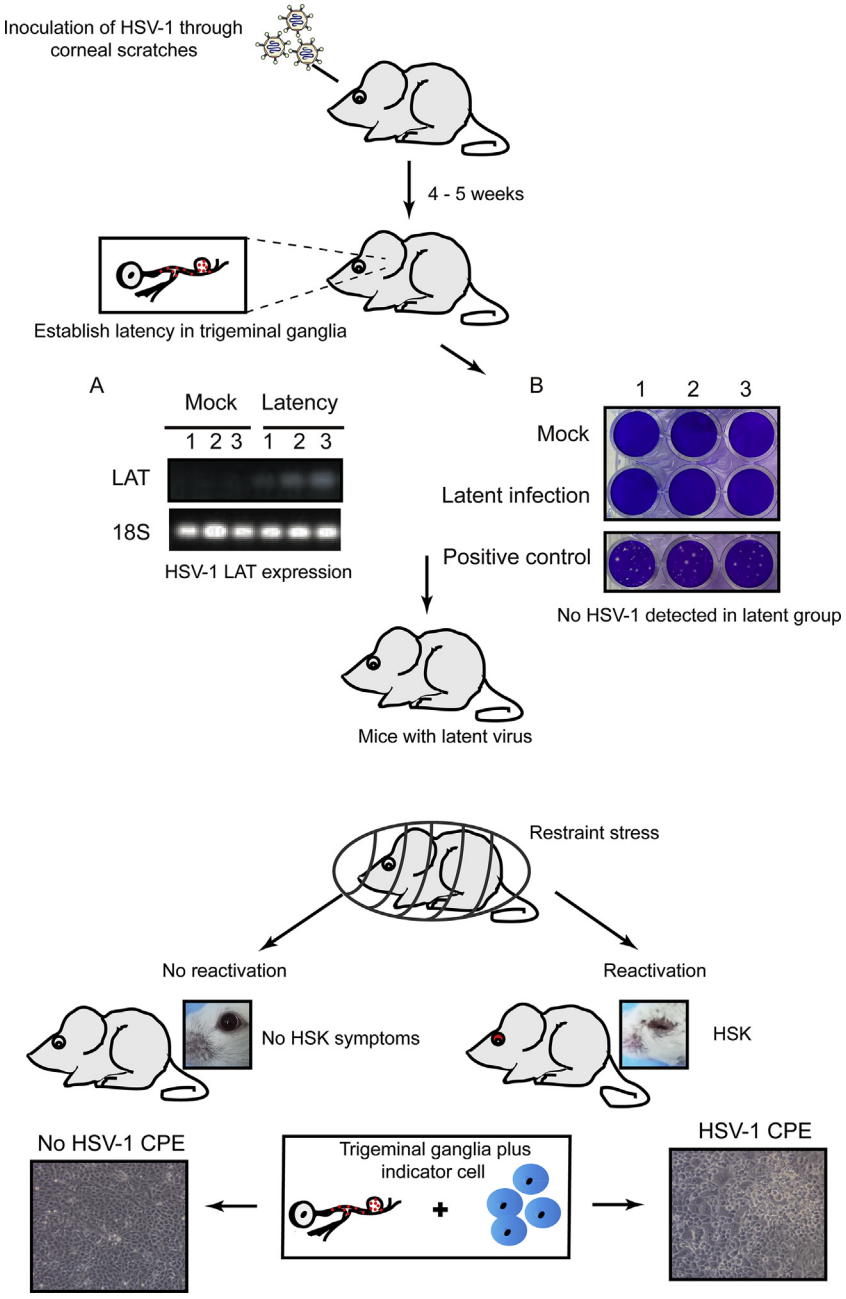

Figure 6 Illustration of restraint stress causing recurrent lesions in mice latently infected with HSV-1. Inoculated with HSV-1 through corneal scratches, the mice were kept under adequate condition for 4−5 weeks to establish latency. When the establishment of latency was confirmed in trigeminal ganglia *via* virus titration measurement, the LAT expression of uninfected and infected groups were tested. Plaque assay showed no detectable productive HSV-1 progeny. The latently infected mice were then loaded with restraint stress, and the phenotype was assessed afterward. Data shown in this figure are unpublished data of our group.

4.3. Stress causes oxidational damage and induces apoptosis

Under UV or physical trauma, dendrite mitochondria produce reactive oxygen species (ROS) to inhibit mTOR activity and decrease the expression of B-cell lymphoma 2 (BCL-2), inducing apoptosis[161]. In order to escape from the soon to be apoptotic cells, HSV-1 spreads among host cells in an attempt to infect new individuals. All viral gene classes will be expressed at the same time and enter full reactivation, followed by the infection of peripheral epithelial and neuronal cells to remain the survival and spread of HSV-1[162]. HSV-1 itself may also be a stimulant to apoptosis. HSV-1 protein ICP27 can increase host cell susceptibility to apoptosis, which is also probably through the production of higher level of ROS[163].

5. Discussion and perspective

With the astonishingly rapid development of industrialization and economics in modern society, people have suffered much more stress caused by the environmental, psychological and physical

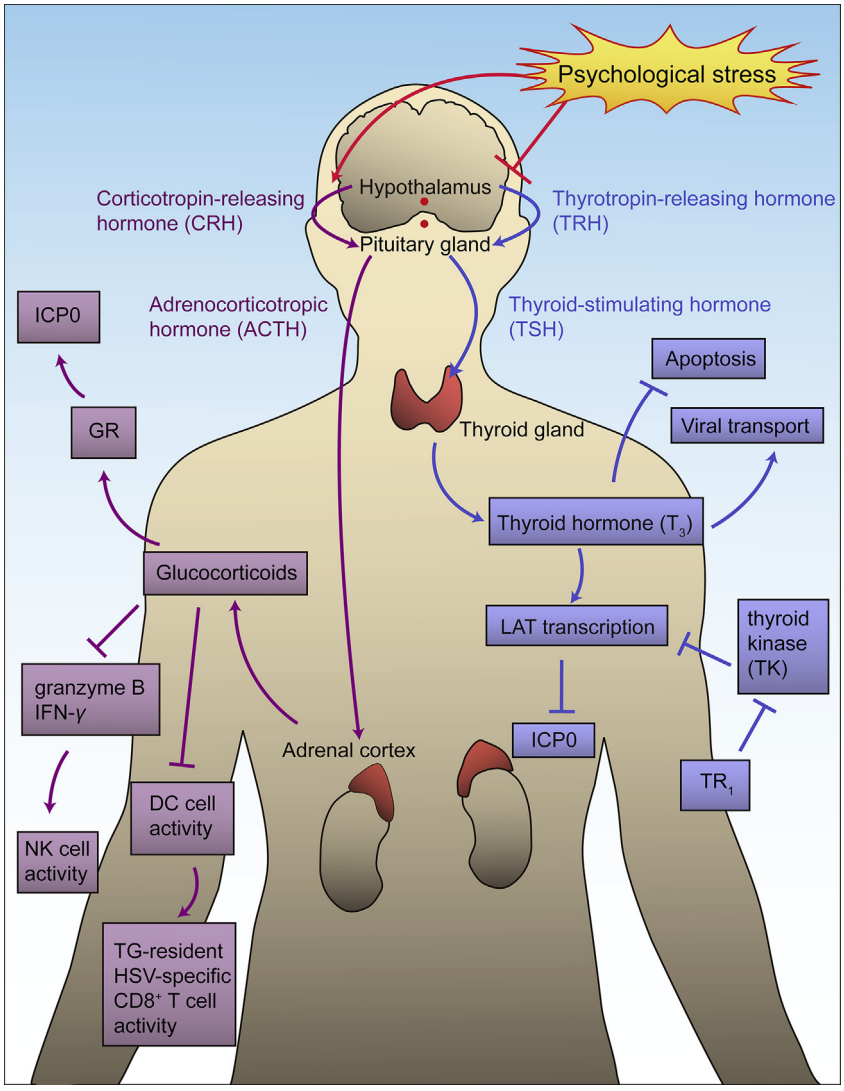

Figure 7 Stress-induced hormone imbalance which leads to reactivation. Psychological stress acts on hypothalamus, stimulates HPA axis (purple arrows) and suppresses HPT axis (blue arrows). In HPA axis, CRH released by hypothalamus increases, enhancing the ACTH released by pituitary gland. As the ACTH level increases, glucocorticoid excreted by adrenal gland also increases. The increased glucocorticoid then activates GRs, activating the transcription of lytic gene *ICP0*. It also inhibits DC activity, causing the reduction of TG-resident HSV-specific $CD8^+$ T cell activity, affecting adaptive immunity towards HSV-1. Since glucocorticoid is able to inhibit granzyme B and IFN-γ secretion, the granzyme B and IFN-γ level are reduced significantly, which causes the decrease of NK cell activity, affecting innate immunity towards HSV-1. In HPT axis, TRH released by hypothalamus decreases, reducing TSH released by pituitary gland. Thyroid gland then reduces the excretion of T_3, which decreases the LAT transcription activated by T_3. The suppressive effect of LAT to ICP0 transcription is weakened, and the increased ICP0 level enhances lytic gene transcription. The suppressive effect of T_3 towards *TK* gene, an important viral gene for HSV-1 reactivation, is also decreased. Overexpressed TR_1 receptor is able to increase the effect of T_3, reversing the changes caused by the reduction of T_3.

factors. Stress, especially psychological stress caused by emotional stimulation, is able to increase the susceptibility and severity of infectious diseases and to cause the reactivation from latency, leading to disturbance on life quality. Therefore, reducing the effect of stress on diseases is becoming an urgent and widely concerned topic. Emotional stress causes internal damage called "Qi-Qing Nei-Shang" and consequently disturbs the Yin−Yang balance in the organism, leading to increased susceptibility to HSV-1 and the recurrent lesions. Nevertheless, how emotional stimulation affects the biological pattern and the nature of HSV-1 reactivation susceptibility still needs further systematic study. Moreover, considering that once an individual is infected, no treatment exists to remove the HSV-1 virus completely from the host, an approach to control latent infection and reduce reactivation becomes the crux of the issue.

In conclusion, it has been well-known that followed by the invasion of HSV-1 into the neurons, the virus travels through microtubules into the nucleus, where most of the HSV-1 genome is silenced due to the specific characteristics of neurons. However, the virus can still function properly to avoid the detection of immune system. Neuronal factors have contributed to the anti-apoptosis effect and facilitated the survival of infected cells. Epigenetic modification and the direct effect of latent gene can reduce lytic gene expression, and thus the virus cannot be detected

by the immune system. The virus can also deplete T cells to reduce the survey intensity. The Yin−Yang balance between the virus-stimulating and virus-inhibiting factors maintains the latency. Under stress, oxidational damage, increased glucocorticoids and decreased thyroid hormones promote the Yang factors and inhibit the Yin factors, consequently disturbing the Yin−Yang balance, leading to productive viral replication.

However, several obstacles remain in the way towards more specific, accurate and coherent understanding of HSV-1 latency and reactivation. Current major animal and cellular models are not sufficient to unravel the mechanistic details. Models of closer genetic similarity with human, *e.g.*, *Rhesus macaques*, and newly developed models like tree shrews may be more adequate models to study HSV-1 latency and reactivation[4,164]. In addition, we still know very little about the molecular mechanism of other stress hormones such as epinephrine, growth hormone and prolactin[33,165]. More investigations on other stress hormones are required for a better overall understanding on stress and HSV-1 reactivation. Additionally, according to a research by Edgar et al[166], herpes virus infection is enhanced under circadian clock disruption stress, suggesting a new possible direction on how stress influences the susceptibility of HSV-1 infection. According to our current understanding on the role of stress in HSV-1 infection and the instruction of TCM theory, many small molecules with potential anti-HSV-1 activity have been discovered from TCMs. We have recently published a review specifically focusing on the anti-HSV-1 small molecules originated from different TCMs, and their pharmacodynamics mechanisms[167]. Therein, three main mechanisms were discussed, including autophagy regulation, immunity enhancement, and inhibition of HSV-1 virus replication and infection processes. Numerous sources, *e.g.*, Lychee flower, *Houttuynia cordata*, and *Curcuma longa* L., and their effect corresponding mechanisms were discussed in detail. They are promising drug candidates for novel treatments to prevent stress-induced susceptibility to HSV-1 and the following recurrent diseases. Furthermore, the combination of Yin−Yang theory and modern molecular biology may create novel perspectives and approaches in new drug discovery and treatment development. Both Yin and Yang factors we demonstrated above are possible targets for anti-HSV-1 drug and treatment development, *e.g.*, the enhancement of Yin factors including HSV-1-specific miRNAs, thyroid receptor β1, and ND10 nuclear bodies, and the inhibition of Yang factors including the expression of glucocorticoids, the cooperation of GR and KLF15, the activity of SGKs, the expression of ICP0 and ICP34.5. More systematic researches based on Yin−Yang theory and other TCM theories, combined with genomics, proteomics, metabolomics, high throughput *in silico* screening, etc., may be available approaches to unlock the treasure house of TCM therapy against HSV-1[168−170]. Further detailed explorations are required for a more thorough understanding of stress-induced HSV-1 susceptibility and reactivation and feasible treatments.

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Author contributions

Rong-Rong He conceived the concept and the framework. Chang Yan collected and analyzed all the data. Chang Yan and Luo Zuo drafted the manuscript. Wen Li contributed to the original data. Xue Li, Robert Dallmann, Yi-Fang Li revised the manuscript. Hiroshi Kurihara and Rong-Rong He revised and approved the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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