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The application of a herpes simplex virus Type I vector for transgenic therapy

Chunfu Zheng*

Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, Canada

Abstract

The key to successful gene therapy is to effectively transfer genes to target cells and control the effective expression of genes to avoid expression in non-target tissues. At present, there are two common methods of gene therapy: non-replicating viruses and viral vectors. The use of non-replicating viruses as vectors often limits the efficiency of gene transfer and is unsatisfactory for clinical application. In contrast, replicative viral vectors can deliver genes from a small number of cells to neighboring cells, significantly improve the efficiency of gene delivery, and stably and persistently express exogenous genes. As a result, the investigation and application of viral vectors are recommended. Herpes simplex virus Type I-derived vectors have gradually become important biological treatments due to their wide host range, integration of long fragments of exogenous DNA, neurotropism, and various animal models.

Keywords: Herpes simplex virus Type I; Gene therapy; Vectors; Transgenic technology

***Corresponding author:**
Chunfu Zheng
(chunfu.zheng2@ucalgary.ca)

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1. Introduction

Herpesvirus is a type of virus that is widely distributed in nature. Most animals carry at least one herpesvirus in their body, and therefore, the host is often found to carry several different herpesviruses at the same time. Herpesviruses seldom infect more than one type of host naturally; therefore, more than 200 herpesviruses are likely to be identified. At present, eight human herpesviruses have been identified, which can be divided into three subfamilies: α-herpesvirinae (herpes simplex virus [HSV]-1, HSV-2 and varicella-zoster virus [VZV]), β-herpesvirinae (human cytomegalovirus HCMV, human herpesvirus types 6 and 7) and γ-herpesvirinae (Epstein-Barr virus and human herpesvirus 8 [Kaposi's sarcoma-associated herpesvirus]).

The herpesvirus family has four significant biological characteristics: (1) these viruses encode a large number of enzymes related to nucleic acid metabolism, DNA synthesis and post-translational modification of proteins, although different species of herpesviruses may encode different enzymes. (2) The synthesis of viral DNA and the assembly of capsids are completed in the nucleus, but the final assembly of virions occurs in the cytoplasm. (3) Destruction of infected cells often accompanies the production of infectious progeny viruses. (4) All herpesviruses detected thus far can cause latent infection in natural hosts. When the virus undergoes latent infection in cells, the viral genome appears in a closed circle form, and only a small portion of viral genes are expressed. The genome of the latent virus retains its ability to replicate and can cause

disease through reactivation. However, the latent infection of viruses and the precise molecular mechanisms involved in the progression from latent infection to reactivation are still unclear, and this mechanism may differ among different viruses.

Latent infection is different from chronic infection in that there are no infectious progeny. Conversely, this reactivation ability differentiates latent infection from abortive infection. Latently infected viruses can simultaneously be latent in some cells and actively multiply in other cells. Therefore, there are several different situations: (1) the virus is essentially latent in all cells (this seems to be the case for VZV; the possibility is low for HSV); (2) in some of the infected cells, the virus undergoes lytic activation, but there are no associated symptoms (such as asymptomatic virus shedding in HSV, which is a common phenomenon for all herpesviruses, including VZV); and (3) lytic replication or proliferation of the virus, which is the essential cause of the disease (some cells remain in the latent state).

HSV-1, the first human herpesvirus discovered, has been intensively studied. Its biological properties are attractive, especially the following characteristics: (1) it can cause a series of infections; (2) it can cause a lifelong latent infection in the host; and (3) reactivation can cause damage at or near the initial infection. Herpesviruses can be used as research models and research tools to investigate intercellular protein shuttling, synaptic connections in the nervous system, membrane structure, gene regulation, gene therapy, cancer treatment and many other biological problems (including the general biological characteristics of other viruses and the specific biological characteristics of HSV).

Since the release of the HSV-1 genome sequence in 1988,[1] many scientific researchers have focused on the effects of the HSV-1 gene on viral replication and pathogenicity. After more than 20 years of hard work, many genes involved in the regulation of viral gene expression, host protein interactions, and evasion of host immunity have been identified.

As an important viral family that infects humans, HSV-1 can infect all human tissues and establish latent infection in the host.[2,3] HSV-1 is a complex neurotropic virus that can be transmitted antegrade or retrograde through the synapses of neurons and can establish a stable latent infection in neurons without affecting their function. To date, HSV-1 vectors have been developed into viral vectors that effectively introduce exogenous genes into the nervous system and are gradually being applied for the treatment of various diseases, including tumors.

2. Biological characteristics of HSV-1

HSV is a typical representative of *Herpesviridae* and has two serotypes, HSV-1 and HSV-2. The HSV-1 viral particles were spherical with a diameter of 100 nm. As shown in Figure 1A, the double-stranded DNA genome and viral DNA-binding proteins together constitute the viral core, which has a diameter of approximately 75 nm. The capsid protein is wrapped around the core, and 162 capsomeres form an icosahedral three-dimensional symmetric structure. The core and capsomere form the capsid. On the outside of the nucleocapsid is an asymmetric and homogeneous tegument that tightly surrounds the capsid. The cortex contains at least 20 viral proteins, some of which are very important for the transcription initiation of viral DNA and the replication of viral particles. The cortex is an envelope that contains various lipoproteins and has various protrusion structures; they are glycoproteins encoded by the virus. At present, 13 different viral glycoproteins have been found to be involved.[4] Once the membrane of the viral particle is removed, it enters the host cytoplasm and reaches the nuclear membrane along tubulin, after which the viral DNA is injected into the nucleus through the nuclear pore.

The HSV genome is a double-stranded linear DNA sequence with a length of approximately 152 kb. The HSV genome is replicated in a rolling circle in the host cell to form an end-end concatemer DNA. As shown in Figure 1B, the genome is mainly composed of two covalently linked fragments, *that is*, a long fragment (UL) and a short fragment (US). These regions are flanked by the short inverted repeat sequences TR_L-IR_L and IR_S-TR_S, respectively. In addition, at the two ends of the double-stranded DNA and the junction of $_{IRL\text{-}IR\ S,}$ there is a single copy of "a," which contains the packaging and entry signals of viral DNA.

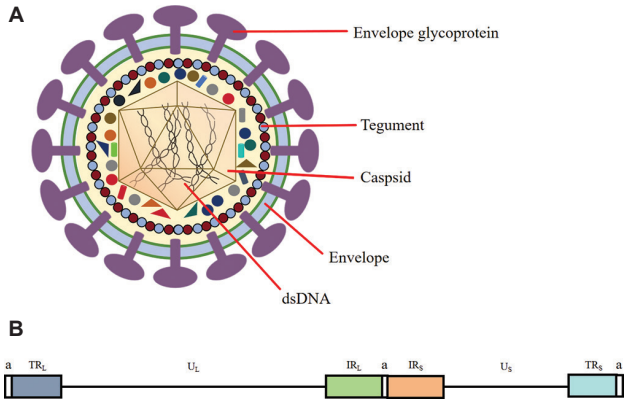

Figure 1. Schematic diagram of the herpes simplex virus (HSV) (A) structure and (B) DNA structure

The timing of HSV-1 transcription and translation are tightly regulated. The viral genome encodes more than 80 viral proteins throughout the entire cycle. Proteins can be divided into three phases based on their expression kinetics during the viral replication cycle: immediate early (IE, α), early (E, β), and late (L, γ) phases. Viral replication, transcription, and nucleocapsid production all occur in the host cell nucleus. After viral DNA is released and enters the host cell nucleus, the genome is circularized, and the cortical protein VP16 interacts with host-related factors and then binds to the enhancers of the five IEs (ICP4, ICP27, ICP0, ICP22, and ICP47) of the viral genome (TAATGARAT), thus initiating transcription.[5,6] The expression products of IE genes directly affect the transcription of the E gene. The E gene encodes mainly enzymes and DNA replication-related proteins. The last is the expression of gene L, which mainly encodes structural proteins required by some viral particles.

HSV proteins include viral structural proteins and non-structural proteins. The former includes glycoproteins and capsid proteins, and the latter includes enzymes encoded by viral genes, such as DNA polymerase, thymokinase, and DNA replicase-helicase. There are other coat proteins located between the envelope and the capsid. In addition to proteins, viral particles also contain spermidine, spermine, and a small amount of proteins and lipids from host cells.

Viral genes can also be divided into essential genes and non-essential genes based on whether they are essential for viral growth. These essential genes are essential for viral replication in cultured cells and account for approximately half of the viral genes, including capsid proteins, viral DNA replication proteins, viral DNA cleavage/packaging proteins and some cortical proteins. Non-essential genes are not completely required for viral replication in cultured cells, but some non-essential genes may also be essential for viral growth based on different hosts, pathogeneses and latent states.

2.1. HSV-1 replication

The propagation process of HSV mainly includes viral adsorption, membrane fusion, invasion, capsid, protein synthesis, DNA replication, nucleocapsid assembly, envelope formation, and mature release of virions.[7]

The binding of some glycoproteins on the viral surface to host cell receptors is called adsorption. HSV-1 infects two main types of cells: epithelial cells and neurons. Some specific envelope glycoproteins can recognize host cell-surface receptors to achieve specific adsorption. After HSV-1 is adsorbed on host cells, it soon enters the cells through membrane fusion, which is the fusion of the viral capsule and the cell membrane. HSVs that enter cells through membrane fusion can complete the replication cycle and initiate infectious proliferation. After the virus enters the cell, the nucleocapsid is delivered to the nuclear pore, and the viral DNA is released into the nucleus with the assistance of a variety of viral proteins.

Under the control of RNA polymerase II and viral proteins in infected cells, HSV-1 genes begin to be transcribed and translated, and proteins are synthesized according to the strict rules of IE gene transcription and expression → production of the IE gene product → activation and regulation of E gene transcription and expression → production of the E gene product → activation and regulation of the transcription and expression of the L gene → generation of the L gene product in this order. The synthesis of most viral proteins requires post-processing modifications for their biological functions, including phosphorylation, glycosylation, ADP ribosylation, and nucleotidylation. The synthesis of viral DNA occurs in the nucleus and requires the participation of many enzymes. Viruses have two main replication modes: theta replication and delta replication.[8] The combination of these two replication modes makes full use of the characteristics of the viral genetic structure to enable it to make full use of the host's limited space and time resources to replicate the progeny viruses as efficiently as possible.

The assembly of the HSV nucleocapsid occurs in the nucleus through three processes: capsid formation, DNA processing and capsid packaging. The assembled virus must first escape from the nucleus to the cytoplasm. In this process, it buds through the nuclear membrane and undergoes the first outer membrane wrapping process. After reaching the cytoplasm, the outer membrane formed by the first encapsulation must be removed, and the process of tegumentation must be completed so that a layer of tegument is formed on the outside of the nucleocapsid. The virus then crosses the plasma membrane again and escapes from the cell through a second outer membrane wrapping process to form the final mature viral particle. This complete process involves the activities of a variety of organelles and a series of biological functions in the cell.[9]

2.2. HSV-1 infection and resistance to environmental and physicochemical factors

Humans are the only natural hosts of HSV-1. The virus enters the body through the respiratory tract, oral cavity, and damaged skin and is latent in the sensory ganglion cells, with most cases being asymptomatic. When the body's resistance, such as fever, focal infection, gastrointestinal dysfunction, and emotional changes, decreases, latent HSV-1 in the body is activated to cause related diseases. When HSV-1 infects healthy people,

they can rapidly develop immunity, and the lesions are limited to only the skin and mucous membrane surfaces. Severe HSV-1 reactivation is often induced in people with deficient immunity (organ transplantation, patients receiving immunosuppressants and antitumor therapy) and those with immature immune function. After neonates are infected with HSV-1, lesions, such as those in the brain, liver, eyes, adrenal glands, lungs, and skin and mucous membranes, often spread throughout the whole body, resulting in severe conditions and high mortality. When extensive skin injury occurs, such as eczema and burns, HSV-1 often manifests as generalized herpes on the skin and mucous membranes and can be transmitted throughout the body to cause disease.

HSV-1 can adapt to a wide range of host cells and can reproduce in various passages of cells with obvious cytopathy. HSV-1 has a wide host range; except in humans, it can infect almost all kinds of epithelial cells and fibroblasts from embryos and neonatal animals. The replication cycle of HSV-1 is 12 – 18 h in most sensitive cell lines, and cytopathic cells appear 24 – 48 h after infection.

HSV-1 is sensitive to heat and dryness and can be inactivated in a 50% humid heat environment or a 90% dry environment for 30 min. The median lethal period of ultraviolet (UV) light is 5 – 7 s, and 2 min of X-ray exposure at 1200 rad can inactivate 90% of the virus. The viruses were inactivated after treatment with 1% phenol (phenol, phenolic acid) for 3 days at room temperature and then treated with 5% phenol at 4°C for 18 h. Treatment with ethanol, ether, or chloroform at 4°C for 18 h can inactivate HSV-1 in the liquid but has little effect on the virus in a dry environment. The viruses were inactivated by treatment with 1:1000 potassium permanganate for 1 h at room temperature. At 35°C, 0.01 mol/L formaldehyde or virus at 100 – 1000 pfu/mL (pfu: plaque forming unit) can be completely inactivated by treatment with 0.001 mol/L iodine for 1 h.

HSV-1 is stable in solution with protein, so 10% horse serum or rabbit serum, 0.1% egg yolk or 0.5% gelatin are commonly used to preserve the virus. When cysteine is present in a viral suspension, it can prevent the oxidation of the virus and protect the virus. Virus-infected tissues can be stored in 50% glycerol at 4 – 8°C for half a year. The HSV can be stored for several years after lyophilization.

2.3. Pathogenicity and immunity of HSV-1

HSV-1 infection is very common and has a global distribution. Humans are the only natural hosts of HSV-1, which is transmitted mainly through direct and close contact. Viruses can invade the body through various channels, including through the mouth, respiratory tract, and damaged skin. Serological surveys have shown that the level of HSV-1 antibody is directly related to age and socioeconomic status. In most underdeveloped countries, 90% of people over 30 years of age are positive for HSV-1. Approximately 50 – 60% of the middle-class population in the United States has detectable levels of antibodies to HSV-1, while that of the group with poor socioeconomic status is close to 90%. After the first infection in healthy humans, patients usually have no clinical symptoms and then develop a latent infection. HSV-1 causes mainly pharyngitis, cold sores, and keratoconjunctivitis.

2.4. HSV-1 infection

HSV-1 relies on the binding of glycoproteins on the envelope to host cell-surface receptors so that the envelope fuses with the cell membrane of infected cells, the cortex and capsid then enter the cytoplasm, and the capsid docks in the nucleoporin complex. The viral DNA is injected into the nucleus with the help of nucleoporin, and then, the replication of new viral DNA and transcription of mRNA is performed in the nucleus. The new virus buds through the nucleolus to form an envelope and then buds via similar protein transport to become a mature progeny virus.

2.4.1. Acute infection

In HSV-1-infected cells, virus-specific gene products are tightly chronologically regulated. IE proteins synthesized 2 – 4 h after infection may play a regulatory role in viral transcript levels. The synthesis of the E protein inhibits the synthesis of the IE protein while it induces the synthesis of the L protein. Late proteins are generally synthesized 12 – 15 h after viral infection of cells and are components of viral particles. The local infiltrating cells of viral infection are initially neutrophils, followed by mononuclear cell infiltration.

HSV-1 is able to spread through the central ganglia or networks of cells innervated by axon nerves and lurks in the sensory and autonomic ganglia.

2.4.2. Latent versus active infections

The virus enters the cell bodies of sensitive ganglia from peripheral ganglia through axonal transport and then undergoes lytic or latent infection.[10] HSV-1 was demonstrated to be latent in the trigeminal ganglion, superior cervical ganglion, and vagus ganglion and occasionally in the dorsal sensory nerve roots of S2-3, as demonstrated by coculture. In the latent infected state, the genome of the virus is encapsulated by histones but does not integrate into the host genome.[11] In this state, the virus enters a relatively quiescent state; almost none of the viral genes are transcribed, and only some viral RNAs associated with latency (LAT and miRNA) are still transcribed.

Patients in the HSV-1 latent state are often activated and transported to terminals along synapses when exposed to sun exposure, stress, trauma, UV, or other infections, where they enter the innervated skin and mucous membranes to reproduce and cause pathological changes again, resulting in the recurrence of local herpes.[12,13] Only in rare cases do viral particles enter the central nervous system (CNS) and initiate latent or lytic infections, which cause symptoms such as encephalitis.[14,15] The newly replicated virus continues to progress, usually at a local site of neural control, to form stable herpes, with a remission period of 2 – 10 days until recovery. In this process, the patient's immune system acts to invade the virus.[16]

2.5. Clinical disease

2.5.1. Acute herpetic gingivitis

It is the most common primary infection. Acute onset, fever, oral and tongue mucosa and gingiva redness, congestion, and groups of herpes, rupture after the formation of small ulcers, ulcers can extend to the throat, palate, *etc.*, occasionally affect the esophagus and trachea, patients with mouth and tongue pain, cervical lymphadenopathy and tenderness and other symptoms. In general, it resolves spontaneously after 1 – 2 weeks.

2.5.2. Acute herpetic nervous system infection

Acute herpetic nervous system infection is rare and presents with encephalitis, meningitis, myelitis, and radiculitis. Herpetic encephalitis is dangerous, with a mortality rate of approximately 50%, and survivors mostly have permanent sequelae.

2.5.3. Keratoconjunctivitis herpeticum

Acute and active infections occur in the eye, herpes is seen locally in the bulbar conjunctiva, and secretions are scant. Lesions in the cornea, visible corneal surface dendritic ulcers, and deeper ulcer healing can cause corneal leukoplakia, seriously affect vision, and can also cause hypopyon. Iridocyclitis and uveitis can cause blindness.

2.5.4. Skin infection and herpetic paronychia

HSV-1 skin infection occurs in skin damage, such as eczema, burns, or trauma, causing eczema herpeticum, herpetic paronychia, and so on. HSV-1 infection, which occurs on the basis of skin eczema, is called eczema herpeticum. Eczema herpeticum and skin burns can cause systemic infections, which are severe and cause a high mortality rate. Herpetic paronychia is often misdiagnosed as bacterial infection after skin damage to the fingers, infection of the fingers or nails, painful herpes, and formation of pustules.[17] Herpes occurs mostly in the local skin, herpes around the skin congestion, herpes dry scab and recovery.

2.5.5. Systemic disseminated infection

Systemic disseminated infection often presents with herpetic lesions such as brain, liver, lung, eye, adrenal gland, and systemic mucocutaneous lesions, and affected patients will experience high fever, cough, dyspnea, cyanosis, jaundice, convulsions, coma, and systemic mucocutaneous herpes, with a mortality rate of 60% –o70%.

Once HSV-1 infects immunocompromised individuals, its lesions can spread to the respiratory tract, digestive tract, intestinal mucosa, *etc.*, and the symptoms are more serious. Sporadic fatal encephalitis caused by HSV-1 infection progresses rapidly and has a high mortality rate.[3]

2.6. Immunity

The clinical symptoms of acute HSV-1 infection tend to be more severe than those of reactivation infection, which is associated with stimulating the body to produce corresponding antibodies and specific T cells after HSV-1 infection. Neutralizing antibodies directed against HSV envelope glycoproteins are important for host protection, and such antibodies can also mediate antibody-dependent cell-mediated cytotoxicity and limit the spread of early HSV-1 infection. Neutralizing antibodies appear in the serum approximately 1 week after infection, peak at 3 – 4 weeks, and persist for many years.

Specific cytotoxic lymphocytes (TCs) detected during the 2nd week of HSV-1 infection can destroy infected host cells before viral replication is complete. Infection with HSV-1 is able to prevent reinfection with HSV-2 or reduce the damage caused by it. In addition, severe HSV-1 reactivation is often induced in immunodeficient individuals, especially immunocompromised individuals, which may be associated with prolonged viral release and persistent damage.

3. Viral diagnosis

3.1. Virus isolation and culture

Viral isolation and culture are the "gold standards" for confirming HSV-1 infection. Blister fluid, saliva, cerebrospinal fluid (CSF), corneal scrapings, and other samples can be inoculated into susceptible cells, such as human embryonic kidney, human amniotic membrane, or rabbit kidney, and can also be inoculated into the chicken embryonic chorioallantoic membrane, and suckling mouse or mouse brain, all of which can increase the isolation rate. Cytopathic effects caused by HSV-1 often appear after 2 – 3 days, and the cells may show pathological features such as swelling, rounding, increased refraction, and the formation of confluent cells. Viruses can be identified and typed by immunofluorescence or nucleic acid hybridization via

HSV-1 monoclonal antibodies and HSV-1-specific nucleic acid probes.

3.2. Rapid diagnosis

After the tissue cells of the oral cavity, cervical mucosa, skin, cornea, *etc.* are smeared, viral antigens are detected through immunohistochemical staining or indirect immunofluorescence with specific antibodies. Wright–Giemsa staining microscopy revealed that HSV-1 infection could be considered if intranuclear inclusions and multinucleated giant cells were found; negative staining of herpes blister fluid by electron microscopy could rapidly confirm the diagnosis.

In situ, nucleic acid hybridization and polymerase chain reaction (PCR) can be used to detect viral DNA. Southern blotting was used to determine the specificity of the PCR products, and PCR amplification of cerebrospinal fluid was considered the best means of diagnosing herpetic encephalitis. In addition, DNA digestion patterns can also be used for HSV-1 identification and type analysis.

HSV-1 antibody determination is of little significance for clinical diagnosis and is only used for epidemiological investigation and diagnosis, and the commonly used method is enzyme-linked immunosorbent assay.

3.3. Prevention principle

Avoiding contact with patients or administering specific antibodies to susceptible people can reduce the risk of HSV-1 transmission. Conventional antiviral drugs act directly against viral DNA polymerase, so it is difficult to remove latent viruses. Acyclovir (ACV), valacyclovir, and famciclovir can inhibit HSV-1 reactivation after infection. Several HSV-1 vaccines (inactivated virus vaccines, subunit vaccines, live attenuated vaccines, live vector vaccines, DNA vaccines, *etc.*) are in different stages of development.

4. HSV-1-derived vectors

The genome sequence of HSV-1 is fully understood, and the molecular mechanism of viral replication is relatively clear, with viral DNA containing at least 37 essential genes. The Us region has only one essential gene, gD, so that it can be replaced by foreign genes,[18] and the modified genome can carry 4 – 50 kb of foreign genes. It was thus developed as a carrier for the treatment of certain diseases in humans.[19-21] These include: (1) transmission and expression of some human genes in neural cells[22,23]; (2) selective destruction of tumor cells[24,25]; (3) prevention and immunotherapy against tumors[26]; and (4) prevention of HSV and other viral diseases.[27]

Compared with other viral vectors, HSV-1 as a vector has the following advantages: (1) it has a wide host range, infecting almost all cell lines; (2) it infects both replicating and non-replicating cells, such as neural cells; (3) it can integrate long exogenous DNA; (4) it is regulated by antiviral drugs, such as ACV or gancyclovir (GCV); (5) it is stably present as appendages in neural cells and can continuously express exogenous genes; and (6) mice, pigs, and monkeys can be selected as model animals for research.

With the development of many new technologies, scientists have used various means to engineer the HSV-1 genome so that exogenous genes can be effectively transmitted and appropriately expressed, greatly reducing harm to the body. Notably, British scientists have been working on the use of herpes virus vectors to treat cancer for many years and have made remarkable achievements in recent years. They successfully treated head and neck cancer and reported that the use of a combination of radiotherapy and chemotherapy to treat cancer is significantly better than the use of monotherapy.[28]

There are three main classes of derivative vectors for HSV-1, as shown in Figure 2: (1) amplicon vectors (amplicon vectors), (2) replication-deficient vectors (defective recombinant vectors), and (3) recombinant attenuated vectors.[29]

4.1. Amplicon vector

Amplicon vectors are defective vectors that require helper plasmids and have relatively high amplification titers that can effectively infect neuronal cells. In terms of structure and immunology, the amplicon vector used is the same as that used for wild-type HSV-1, but it carries multi-conjoined plasmid DNA rather than the viral genome.[30] Amplicons are bacterial plasmids that contain the DNA replication initiation sequence (ori) and DNA cleavage/packaging signal (pac) of HSV-1 in addition to the prokaryotic replicons essential for replication in bacteria and resistance marker genes available for screening but do not contain any other virus-encoded proteins and cannot replicate independently. With the help of helper viruses, the circularized amplicon allows efficient DNA amplification, encapsulates linear DNA as long as 152 kb into HSV-1 particles (Figure 3),[31,32] becomes an infectious pseudovirus containing multicopy tandem plasmids, and ensures that the vector replicates extrachromosomal in infected cells, that is, in the form of episomes, and does not cause insertional mutagenesis or positional effects on host cell chromosomes.[33] The HSV-1 tsk strain is a good helper HSV-1 virus whose replication is temperature-regulated and can multiply at 31°C while helping amplicon vectors replicate and package. When the harvested virions were subsequently cultured at 37°C, the proliferation of HSV-1

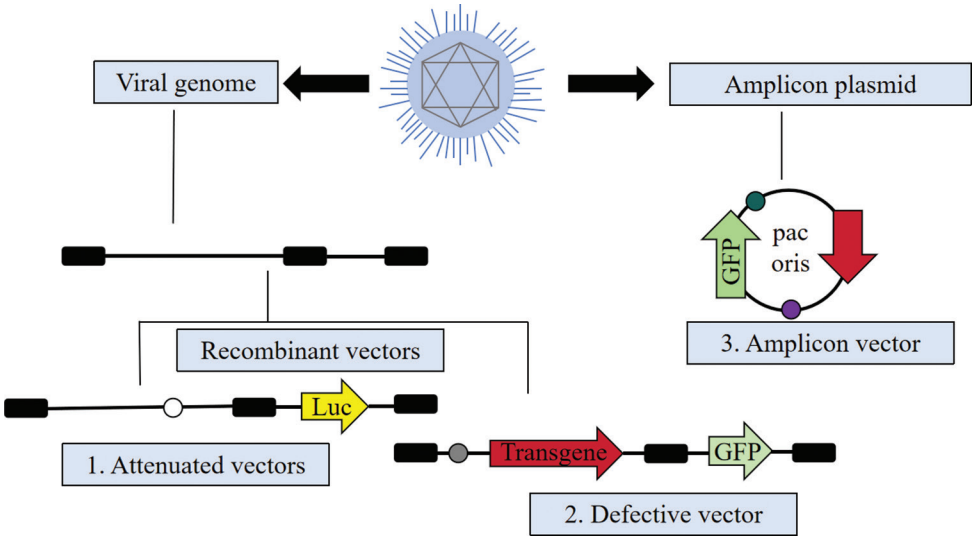

Figure 2. Three herpes simplex virus Type I (HSV-1)-derived vectors

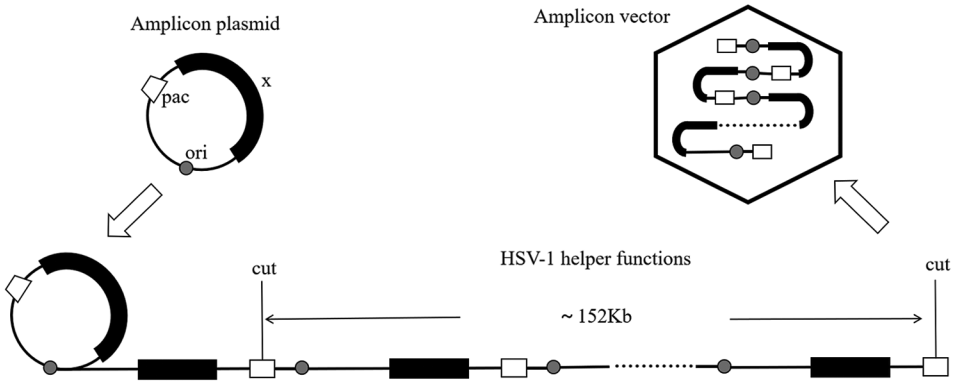

Figure 3. Amplification and packaging mechanism of amplicon vectors
Abbreviation: HSV-1: Herpes simplex virus Type I.

tsk was inhibited, thus preventing cytopathic effects caused by activation of the inapparent infection state.

As a gene delivery tool, amplicon vectors have the following advantages: (1) an extremely large transgenic capacity of approximately 150 kb DNA; (2) amplicons can carry multiple repetitive genes, thereby improving transgene copies in each cell; (3) a wide range of cellular tropisms, which can infect a variety of cells, including dendritic cells (DCs); (4) vectors are easily constructed; (5) low toxicity, which is not pathogenic to the organism; and (6) a very low likelihood of causing insertional mutations.

Given their large transgenic capacity, amplicons have become one of the most interesting, multifunctional, powerful, and promising gene transfer platforms. These vectors can be used to deliver multiple copies of small genes, a series of full-length genes encoding complex structures, or a single-copy gene as long as 150 kb, containing all exons, introns, and large upstream and downstream operator genes. The use of amplicon vectors for gene therapy, although more effective at delivering genes into target cells, is gradually being diluted as cells divide, and its application is greatly limited. Therefore, amplicons are commonly used to transmit genes to the CNS or peripheral nervous system, and HSV-1 has good adaptability in the nervous system environment.

However, even deletion viruses act as helpers and lead to significant cytotoxicity and immune responses, greatly hampering the use of amplicons in gene therapy and vaccine production. Recent technological advances have significantly improved and expanded the use of amplicons in neurology, resulting in improved amplicon vectors that no longer require helper plasmids[34] or carry very few non-pathogenic helper particles.[35] In the original helper

virus-independent packaging system, its helper function is provided by a series of cosmids with overlapping regions carrying the HSV-1 genome (with the pac packaging signal removed).[36] Recently, bacterial artificial chromosome (BAC) technology has been used to clone the entire HSV-1 genome lacking the pac signal into the BAC, which can efficiently replicate DNA in bacteria and effectively exert the function of HSV-1 after entering cells, greatly simplifying the system and significantly improving its efficiency.[37] There is also a Cre/loxP site-specific recombination system that is missing the packaging signal for helper viruses and replicates amplicons equally efficiently.[38] Therefore, amplicon vectors have emerged as very useful tools in neurological studies.[32]

In addition, there are many derived HSV-1 amplicon vectors, such as HSV-1/adeno-associated virus (AAV) hybrid amplicons (Figure 4), which combine HSV-1's ability to carry large volumes of DNA as well as site-directed integration of AAV and sustained expression of transgenes.[39] HSV/AAV amplicons contain most of the properties of the virus, and adeno-associated virus can integrate its genomic DNA into human chromosome 19, thereby increasing its stability. These methods have shown that amplicons can safely deliver large fragments of DNA into the mammalian nuclear environment, turning many years of dreams into reality, and investigators have applied this feature to multiple experimental systems.[32]

Non-helper-dependent amplicon systems have been widely used in many experimental models of gene therapy to study neural dysregulation and vaccine development. As gene transfer vectors, amplicons, such as neuro-factors such as nerve growth factor and brain-delivered neurotrophic factor, anti-apoptotic proteins, heat shock proteins, or antioxidant enzymes, have been used to deliver and express exogenous genes for brain injury therapy.[40,41] Amplicons expressing neurotransmitters or neuroreceptors have also been used in studies of behavioral traits such as learning and memory.[40] However, transgenes are usually expressed transiently, largely due to the loss of non-replicating and unintegrated vector DNA in host cells, and whether amplicon vectors can be widely used must overcome these difficulties. The amplicon production and purification procedures also need further improvement. At present, we still cannot fully understand which viral proteins are involved in regulating gene expression, thus directly affecting amplicon carrier gene expression.

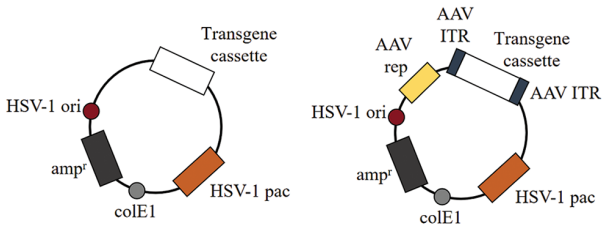

Figure 4. Schematic representation of the HSV-1 amplicon (left) and the HSV/AAV hybrid plasmid (right)
Abbreviations: AAV: Adeno-associated virus; HSV: Herpes simplex virus; ITR: Inverted terminal repeats.

4.2. Replication-deficient vector

Replication-deficient vectors selectively mutate or delete some genes essential for growth in cell culture so that the virus cannot replicate in normal cells unless complemented in trans in transformed cell lines. HSV-1 encodes at least 84 genes, half of which are non-essential. Close to 30 kb in the genome can be replaced by foreign genes, which makes it possible for HSV-1 to carry one to several foreign genes for gene therapy.

Several replication-deficient vectors have been successfully constructed. Deletions were performed in different combinations using different IE genes. The first-generation replication-deficient HSV-1 vector lacking ICP4 was named d120.[42] Although this defective virus reduces pathogenicity and efficiently transmits and transiently expresses reporters in the brain, it is toxic to cultured neuronal cells, limiting its application. The codeletion of five IE genes eliminates cytotoxicity and thus allows the vector gene to be continuously expressed in cells,[43-45] further indicating that the cytotoxicity caused by the first-generation deletion is caused by the other four IEs.[46] In neuronal cells, the deletion allows sustained expression of exogenous genes that are expressed starting with the ICP0 IE promoter or the HCMV IE promoter.[47] In the second generation, ICP4 and ICP27 are commonly deleted.[48] Its advantage lies in the absence of expression of viral early and late genes, providing sufficient space for independent expression of different exogenous genes. As a third-generation HSV-1 viral vector, three non-essential genes, ICP6, ICP47, and γ34.5, are simultaneously knocked out in G47Δ, which has efficient and high biosafety oncolytic ability.[49]

4.3. Replication attenuated vector

Deletion of some non-essential genes allows the virus to replicate efficiently *in vitro*, but replication is blocked *in vivo*. Several viral genes that affect viral replication, cytotoxicity, and immune invasion have been identified, and these genes usually interact with multiple cytokines, thereby optimizing viral replication conditions and inhibiting cell growth. In view of these characteristics, investigators have successfully constructed an HSV-1 deletion that restricts replication in normal quiescent cells and efficiently replicates in both tumor cells and dividing cells.

These attenuated viruses are often used as therapeutic vectors for gene delivery. Attenuated HSV-1 vectors have been applied to live viral vaccines as oncolytic viruses and gene therapy vectors to deliver genes into nerve cells. The toxicity of many HSV-1 non-essential genes is reduced in animal models. Among them, thymidine kinase (TK), ribonucleoside reductase (RR), viral host shutoff protein Vhs (encoded by UL41), and ICP34.5 have been widely studied. TK genes are involved in nucleotide metabolism for viral growth and efficient replication in neurons. RR is required for the conversion of rNTPs to dNTPs in neuronal cells and viral DNA synthesis.[50] Vhs destabilize host mRNAs and limit cellular protein synthesis, thereby providing favorable conditions for viral protein synthesis.[51] During lytic infection, Vhs also leads to viral mRNA instability and prevents the overexpression of IE and E proteins. ICP34.5 acts as a neurotoxic factor and is involved in HSV-1 pathogenicity.[52] It is involved in multiple periods of viral replication and is multifunctional, one of which is preventing cytokine-induced restriction of viral growth. ICP34.5 recruits protein phosphatase Ia and phosphorylates eIF2α again to ensure smooth protein translation as well as viral replication. The PKR pathway is often disrupted in tumor cells, and eIF2α levels increase, allowing the ICP34.5 deletion virus to replicate as well.[53,54] In non-permissive cells, deletion of ICP34.5 leaves the nucleocapsid in the nucleus, and viral replication does not persist.

Newly developed attenuated vectors, in addition to the deletion of these viral genes, also insert genes encoding various cytokines, such as interleukin (IL)-4, IL-12, IL-10, granulocyte/macrophage stimulating factor (GM-CSF) or the costimulatory factor B7-1, thereby maximizing tumor immunogenicity.[28,55-57]

With attenuated HSV-1 as a vector, in addition to genetic stability factors, many safety factors, such as viral latency, reactivation, or recombination with virulent wild-type viruses, are worth exploring.

4.4. Purification and quantification of HSV-1 virus particles

4.4.1. Purification of virions

Amplicon vectors are packaged into infection-competent pseudoviruses with the help of helper viruses. As with the virions amplified from the other two derived viral vectors (replication-deficient and replication-attenuated), wild-type HSV-1 was purified and quantified. To obtain higher titers and pure virions, sucrose gradient centrifugation is usually employed to enrich and purify virions effectively.[58,59] Its operation steps are briefly described as follows:

(1) After the cells infected with the virus showed lesions such as swelling, rounding, and cell fusion, the cells and supernatants were collected;
(2) Cells were repeatedly frozen and thawed at −70°C and 37°C to lyse the cells;
(3) Centrifuge at low speed to collect the supernatants;
(4) Centrifuge at 22,000 rpm for 1 h to remove the supernatants;
(5) The mixture was resuspended in TBS (200 mM NaCl, 2.6 mM KCl, 10 mM Tris-HCl [pH 7.^{5}, 20 mM $MgCl_2$, and 1.8 mM $CaCl_2$), added to different concentrations of sucrose (30%, 40%, and 50%), and gradiently centrifuged at 20,000 rpm for 2 h;
(6) Fluids with sucrose concentrations ranging from 40 to 50% were collected, centrifuged again, and resuspended in TBS;
(7) Store in a −80°C refrigerator or liquid nitrogen tank for future use.

4.4.2. Viral particle titration

With respect to the titration of virions, plaque formation assays are often employed and are capable of detecting infection-competent viruses. In general, monolayers of Vero or other host cells are infected with a range of virion dilutions. The virus will multiply in infected cells, eventually causing cytotoxic effects and release. The released virus will infect neighboring cells. The whole process is repeated continuously, eventually leading to the formation of plaques. To stop the spread of the virus to other areas of the cell and start a new plaque formation process, a layer of soft agar is spread over the surface of the cell after completing the initial infection. Its operation steps are briefly described as follows:

(1) Vero cells were plated in 96-well plates until they were overgrown;
(2) The virus was diluted in a medium gradient. EP tubes can be used for virus dilution, generally with three replicates for each concentration;
(3) The culture medium in the 96-well plate was removed, and 100 μL of virus mixture was added to each well;
(4) After 1 –o1.5 h, the virus mixture was removed, and the tip was changed each time. Another 200 μL of complete medium (containing 2% fetal bovine serum) was supplemented and cultured for 24 – 48 h. Close observation was performed until the number of plaques that appeared at the lowest concentration no longer increased;
(5) The medium was discarded, 30 μL (5% crystal violet + 70% alcohol) was added to each well, and after 2 min of incubation, the wells were repeatedly washed with double-distilled water.
(6) After drying, the plaques were counted, and the titer was calculated.

5. Application of the HSV-1 vector

HSV-1 has the characteristics of neurotropic cells and can establish a stable latent state; thus, it has become a novel viral vector for exogenous genes to enter the nervous system. For more than two decades, research has shown that HSV-1 viral vectors have opened new paths for gene therapy, from theoretical ideas to basic research to clinical exploration. Compared with traditional gene therapy methods and effects, the current research data and results support the application of HSV-1 vectors.

Due to the reliable biosafety of the HSV-1 virus, three HSV-1-derived vectors were used in clinical experiments very early, but basic research is being conducted. For example, amplicon vectors are mainly used for the following reasons: (1) experimental gene therapy for inherited genetic diseases of the nervous system, such as movement disorders; (2) studies on neurodegenerative disorders using experimental models of Parkinson's disease and Alzheimer's disease; (3) studies on neuroprotection and synaptic repositioning; (4) studies on brain tumors; and (5) studies on a series of composite functions of the nervous system caused by anxiety, sexual behavior, learning, and memory in animal models.

Many different types of HSV-1-derived vectors have also entered the preclinical stage, not only for the nervous system but also for muscle, heart, liver, and other tissues that have undergone certain therapeutic studies but also for individual transgenic HSV-1 vectors in the clinical experimental stage. Importantly, talimogene laherparepvec has been approved by the U.S. Food and Drug Administration for the treatment of malignant melanoma. Here, we briefly introduce the application of herpesvirus vectors in oncolytic therapy, vaccine development and chronic pain treatment.

5.1. Oncolytic therapy

Genetically altered replicative viral vectors have been used in cancer therapy and gene delivery. These highly attenuated viruses can selectively invade and kill tumor cells through a process known as oncolytic virotherapy, which has been extensively studied over the past decade. HSV, adenovirus, and vaccinia virus have been engineered for cancer treatment. As a class of oncolytic viral vectors, HSV-1-derived viruses have several obvious advantages: (1) they can infect many types of tumor cells; (2) the DNA genome is large and can be easily modified by modern DNA recombination technology, recombining and adding exogenous therapeutic genes, thereby strengthening the oncolytic properties of the virus; (3) some viruses have begun to be clinically applied to human tumors such as gliomas and have good biosafety; and (4) they have effective drug control.

HSV-1 oncolytic viral vectors rely on the unique highly expressed enzymes TK and ribonucleoside reductase (RR) in tumor cells to initiate and continuously self-replicate, induce an immune killing response, and ultimately lead to the disintegration and death of tumor cells. However, for differentiated and mature normal cells, viruses do not undergo self-replication even when they enter cells due to the lack of the specific enzymes TK and RR. DlspTK is a recombinant HSV-1 strain lacking TK that replicates in a manner dependent on a high content of TK in tumor cells and inhibits tumor growth when inoculated into gliomas in the brains of nude mice. However, the virus strain is still neurovirulent, and TK is the target molecule of the antitumor drugs GCV and ACV; therefore, these two drugs cannot be used for treatment at the same time, which greatly limits the oncolytic application of the virus strain. hrR3 is an HSV-1 recombinant strain that is inactivated by the insertion of the RR gene via the LacZ gene and has therapeutic effects on rat gliomas as well as metastatic colon and pancreatic cancers, but it remains neurotoxic.[60] Recombinant HSV-1 lacking γ34.5 was found to be neurotoxic and to replicate in dividing cells, and recombinant HSV-1 R3616 and R1716 lacking double copies of γ34.5 showed high safety in both mouse experiments and phase I clinical trials. R3616 is used to treat gliomas and ovarian tumors. In mice, R1716 combined with chemotherapeutic agents also showed good efficacy in the treatment of non-small cell lung cancer, breast cancer, malignant mesothelioma and neuroblastoma.

The investigators subsequently developed oncolytic viruses with multiple genetic mutations, mainly deletions of γ34.5, together with one or several IE genes, such as NV1020, G207, and G47Δ. The NV1020 virus, which loses most of the IR region of HSV-1 while inserting multiple envelope glycoprotein-encoding genes of HSV-2 into this locus and retaining a single copy of γ34.5, has now entered Phase II clinical trials. NV1023, modified from NV1020, has a certain inhibitory effect on prostate cancer, head and neck squamous cell carcinoma, and colon cancer, and the fusion ability of OncSyn and OncdSyn can also effectively inhibit the growth of breast cancer in mice.[61] The G207 virus lacks a double copy of γ34.5 on the basis of hrR3, is safe, is very sensitive to GCV and ACV, and can effectively treat malignant gliomas in clinical Ib/II.[62,63]

The G47Δ virus was engineered by knocking out ICP47 on the basis of G207.[64] ICP47 is associated with Type I MHC-mediated antigen presentation in infected cells, and ICP47 deletion induces robust immune effects and kills infected cells. Knockdown of the ICP47 gene was accompanied by knockdown of the promoter of the latter US11 late gene, which was directly controlled by the

ICP47 gene promoter to become an IE gene, improving the self-replication ability of the G47Δ virus[49] and making the G47Δ virus a safer and more effective oncolytic virus. In a mouse tumor model, this virus strain was significantly more effective at inhibiting tumors than G207 was.[64]

In addition, while engineering the HSV-1 virus itself, the investigators constructed a series of oncolytic HSV-1 strains that carry immune activation, tumor therapy, and tumor-targeting genes, thereby enhancing tumor-inhibiting activity and tumor targeting. Several tumor suppressor genes have become the main targets of HSV-1 viral vectors. Todo *et al.*[65] integrated the IL-12 or soluble B7-1 gene into the genome of G207, applied it in clinical trials and achieved good therapeutic results. Ino *et al.*[66] integrated IL-12, IL-18, and B7-1 into the UMGH-1 virus with a genome structure similar to that of the G207 genome, forming four novel MGH-l mutant viruses carrying exogenous genes. Based on the results of the *in vitro* cytotoxicity test and the growth inhibition effect on transplanted tumors, the four viruses were superior to MGH-1 in terms of killing effect. To improve the targeting of virus strains, genes essential for virus replication were placed under the control of tumor-specific promoters or enhancers to proliferate in tumor cells specifically. The ICP4 and γ34.5 genes are controlled by a pancreatic cancer-specific promoter and brain tumor-specific promoter, respectively, and the obtained recombinant viruses target pancreatic cancer and malignant glioma, respectively, and can effectively inhibit tumor growth.[67,68]

Other oncolytic HSV-1 strains arise from spontaneous mutations in the virus. For example, HF10, an oncolytic virus with high safety obtained by clinical screening, has a 3.8 kb deletion (11,6515 – 12,0347 bp) at the junction between UL and UL/IRL in its genome; a 6,025 – 8,319 bp deletion in TRL; and a 110 488 – 116 514 bp DNA fragment in HSV-1 inserted in the reverse direction, preventing the expression of UL43, UL49.5, UL55, and LAT.[69] Two clinical trials have been conducted using the HF10 strain: One in 6 patients with recurrent breast cancer who received HF10 with significant tumor regression and no significant adverse effects[70] and the other in three patients with head and neck squamous cell carcinoma who received HF10 with no significant tumor regression, but a large number of tumor cells died or became fibrotic according to pathological tests.[71] HF10 has also been tested as a helper virus in animal models for tumor suppression.

Trials utilizing HSV-1 amplicons for oncolytic therapy are also ongoing. In rodent and human glial cell lines, cyclophosphamide (CPA) was successfully transformed into a toxic metabolite in tumor cells through the use of the amplicon-expressed fusion protein 4B1-EGFP to combine the advantages of cytochrome protein p450 4B1 (a potentially biologically activated suicide gene) expression. In addition, a strong bystander effect of cell-to-cell contact was clearly observed.[72] Amplicons have also been used to deliver TK and cytosine deaminase to murine 9L gliomas and human Gli36 glioma cells, which were subsequently treated with GCV and 5-fluorocytosine (5-FC).[73]

In addition, oncolytic viruses combined with chemotherapy or radiotherapy have been used in preclinical models. However, the molecular mechanisms underlying this coordinated effect are currently unknown. A widely accepted hypothesis suggests that radiation therapy or chemotherapy enhances the expression level of GADD34 (blocking the cell cycle to G1/G2) and thus improves the replication capacity of the virus, thereby enhancing antitumor effects (Figure 5)[74] The upregulation of GADD34 expression has been reported in HSV-1-infected head and neck cancer, cholangiocarcinoma, and lung cancer cells after radiation.[75-77] Therefore, multiple mechanisms are likely involved in the combined treatment of oncolytic viruses and traditional therapies, and this synergistic treatment approach helps to maximize oncolytic efficiency while reducing treatment-related virulence effects.

In recent years, scientists at the British Cancer Institute have made important progress in using transgenic herpes viruses to successfully treat head and neck cancer.[28] At the Royal Marsden Hospital, 17 lymphoma patients received modified HSVGM-CSF (deletion of ICP34.5 and ICP47, insertion of GM-CSF)[78] while receiving conventional radiotherapy or chemotherapy for cancer. The results revealed that 93% of the subjects experienced no recurrence after resection of the tumor, and 82% of the patients experienced no recurrence after 2 years. Only 2 of the 13 patients who received high-dose transgenic virus therapy relapsed. Knowing that the above-mentioned study is only a small-scale trial, further investigations are still necessary for safety testing and validation of transgenic herpesvirus therapy for clinical use. However, the existing experimental results show that the use of oncolytic HSV-1 therapy to treat tumors has broad potential.

5.2. Vaccine development applications

HSV-1 vectors carrying exogenous antigens as antiviral infection prevention vectors have the following advantages: (1) different inoculation methods can trigger a strong and continuous immune response; (2) viral DNA is free outside the host cell genome in the form of episomes, avoiding the potential safety hazards caused by random integration; and (3) the ability to carry TK genes can combine with specific antiviral drugs and kill virus-infected cells.

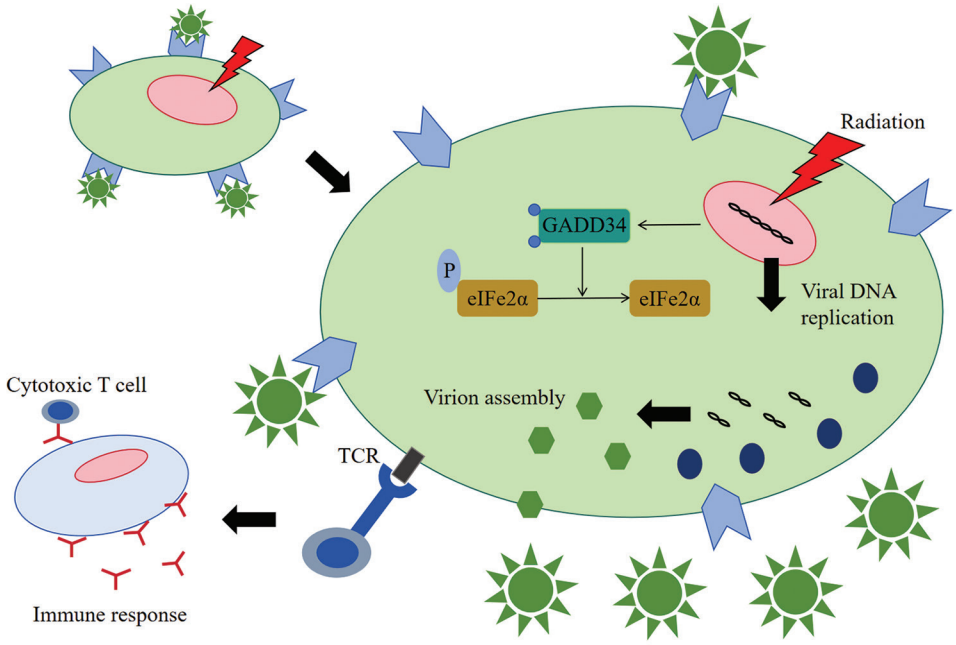

Figure 5. Radiation enhances viral replication and promotes the lysis of tumor cells
Abbreviation: TCR: T cell receptor.

Amplicons have already been used as vaccine vectors for human immunodeficiency virus,[79] intracellular bacteria,[80] and neuropathogenic agents (Alzheimer's disease and prion dysregulation).[36,81] Amplicons have been successfully used to carry all the genes of the mouse leukemia virus (MoMLV) vector and, therefore, rescue retroviruses integrated into the cellular genome,[82] expressing structural proteins[83] or non-structural proteins of Hepatitis C virus.[84] Interestingly, the use of amplicons as carriers of cytokines could constitute a research strategy for cancer vaccines.

Virus replication is necessary for efficient antigens to elicit robust immune responses. However, some non-replicating vaccines, including replication-deficient HSV-1, elicit immune responses.[85,86] These viruses can still infect host tissues but cause relatively low cytotoxicity, which may be related to their inability to replicate and spread in the host. In an antigen model, ICP4, ICP22, and ICP27 null HSV-1 carrying ovalbumin (OVA) protected against lethal effects triggered by OVA expressed by monocytes.[87] In addition, HSV-1, a mutant lacking ICP27 that carries Env and Nef antigens of simian immunodeficiency virus (SIV) and infects rhesus macaques, effectively protects against mucosal destruction triggered by the highly pathogenic SIV mac239.[88]

HSV-1-derived virus d106, which lacks multiple IE genes and has good immunogenicity and protective ability in rhesus monkey models,[27] has been developed into a vaccine vector for the treatment of AIDS and has entered clinical trials. To improve the safety of clinical application, the UL41 gene was further deleted, and the sensitivity of this derived virus to ACV, named d106S, was improved further.[89]

5.3. Treatment of chronic pain

The treatment of cancer pain has long been a medical problem that has an impact on human health and social development and has become the second most common disease after upper respiratory tract infection in North America. Although many types of pain can be managed pharmacologically, the drug affects the nervous system, causing sedation, irritability, anxiety, respiratory depression, and non-neural sites, resulting in urinary retention, constipation, and addiction and tolerance, all of which limit the use of the drug.

Transgenic viral vectors have the advantages of high transfection efficiency and stable and lasting expression of exogenous genes. When a non-toxic replication-deficient viral vector is used, the target gene is introduced into the CNS, which is expected to achieve significant, stable, and lasting expression for treatment.

The HSV-1-mediated proenkephalin gene (hPPE) has been used to treat chronic pain. Wilson *et al.*[90] used a recombinant viral HSV vector carrying the hPPE gene under the control of the HCMV promoter, and immunohistochemical methods revealed that the expression of hPPE in the spinal dorsal horn and dorsal

root ganglion (DRG) inhibited the sensitivity of C-fibers to capsaicin (low stimulation intensity) and/or Aδ to dimethylsulfoxide (high stimulation intensity) stimulation and significantly prolonged the latency of the leg withdrawal response in rats, suggesting that the function of sensory neurons can be altered by the transfection of the target gene with the viral vector. Using a formalin test, they inoculated rats with HSV-1 deletion of the proenkephalin gene (ICP4 removed) in the soles of the feet and reported that vector-mediated enkephalin expression directly produced antinociceptive effects against formalin-induced inflammatory pain stimulation for up to 4 weeks and that reinoculation of the vector again produced antinociceptive effects.

Goss *et al.*[91] injected osteolytic osteosarcoma cells into the bone marrow cavity of the right femur of mice and inoculated them with the hPPE vector 7 days later, and the control group was inoculated with the LacZ-expressing vector; the authors reported that mice inoculated with hPPE had significantly reduced naltrexone-reversible pain-related behaviors, as assessed in terms of open-field motor activity, indicating that HSV-1-mediated gene therapy for hPPE also significantly attenuated nociception in cancer pain. HSV-1-based non-replicative vectors have also been applied to treat pain resulting from Parkinson's disease and spinal cord injury.[92] In addition, the use of these viral vectors to transfer vascular endothelial growth factor into the DRG of mice with diabetic neuropathy is also an efficient treatment.[93]

HSV-1-derived vectors have great importance for treating chronic pain, but gene therapy through these viral vectors is still in the early stage of clinical trials, and a series of problems remain to be solved, such as the neurotoxicity of the viral vector, neuronal lysis caused by viral proteins, selection of the best target gene, stability and targeting of transgene expression, and immune strategies to cope with the host.

6. Summary

HSV-1-derived vectors have already been applied in cancer therapy, gene delivery, and vaccine vectors. Highly attenuated HSV-1 can selectively invade and kill a variety of tumor cells safely and can be effectively regulated by drugs. The successful treatment of patients with head and neck cancer via the use of transgenic HSV-1 vectors combined with radiotherapy and chemotherapy effectively inhibits cancer recurrence after tumor resection surgery. HSV-1-derived vectors have broad application prospects in oncolytic therapy. The HSV-1 vector has a high transfection rate in DC cells, can load multiple antigen genes at the same time, and has very broad potential as a gene vaccine.

Neurological lesions are common, and there are no specific treatments available. With the development of genetic engineering, HSV-1-derived vectors have been gradually applied in the treatment of neurological lesions. HSV-1 can infect post-mitotic neurons, retrogradely enter the CNS after infection of peripheral nerves, and establish latent infection; thus, HSV-1 can be used as a vehicle for exogenous genes.[94-96] HSV-1 neurotoxicity mainly stems from non-essential genes, so investigators have used genetic engineering technology to knock out these non-essential genes to attenuate or ameliorate HSV-1 neurotoxicity.

HSV-1-derived viruses are poorly initiated and can only be expressed for short periods in neural cells through exogenous promoters. Investigators are working to construct a model that uses latency-associated transcript promoters to express exogenous genes by investigating latency in the viral life cycle.[97] HSV-1-derived vectors are very important for treating neurological diseases such as chronic pain, but they are still in the early stage of clinical trials, and a series of problems, such as the stability and targeting of transgene expression and immune strategies to cope with the host, need to be solved.

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Conflict of interest

Chunfu Zheng is an Editorial Board Member of this journal but was not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. The author also declared that he has no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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