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Review Article

Presage of oncolytic virotherapy for oral cancer with herpes simplex virus

Yoshiaki Yura*

Department of Oral and Maxillofacial Surgery II, Osaka University, Graduate School of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan

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Summary A virus is a pathogenic organism that causes a number of infectious diseases in humans. The oral cavity is the site at which viruses enter and are excreted from the human body. Herpes simplex virus type 1 (HSV-1) produces the primary infectious disease, gingivostomatitis, and recurrent disease, labial herpes. HSV-1 is one of the most extensively investigated viruses used for cancer therapy. In principle, HSV-1 infects epithelial cells and neuronal cells and exhibits cytotoxicity due to its cytopathic effects on these cells. If the replication of the virus occurs in tumor cells, but not normal cells, the virus may be used as an antitumor agent. Therefore, HSV-1 genes have been modified by genetic engineering, and *in vitro* and *in vivo* studies with the oncolytic virus have demonstrated its efficiency against head and neck cancer including oral cancer. The oncolytic abilities of other viruses such as adenovirus and reovirus have also been demonstrated. In clinical trials, HSV-1 is the top runner and is now available for the treatment of patients with advanced melanoma. Thus, melanoma in the oral cavity is the target of oncolytic HSV-1. Oncolytic virotherapy is a hopeful and realistic modality for the treatment of oral cancer.

Contents

* Fax: +81 0742 35 6824.
E-mail addresses: narayura630@gmail.com, narayura@m3.kcn.ne.jp

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1. Introduction

Head and neck cancer accounts for 6% of all malignancies worldwide. Oral cancer is included in head and neck cancer, is the fifteenth most common malignancy globally, and accounts for approximately 1% of all malignancies in Japan. Most cases of oral cancer are squamous cell carcinoma (SCC). Surgery, radiation, chemotherapy and the combination of these modalities are common therapeutic methods to treat patients. The therapeutic effects of chemoradiotherapy using cisplatin markedly improved 5-year overall survival over radiotherapy alone; however, survival rates in advanced cases are still low [1,2]. A recent aspect is the introduction of molecular target therapy using an antibody against the epidermal growth factor receptor (EGFR), cetuximab. The treatment of locoregional advanced head and neck cancer with concomitant high-dose radiotherapy plus cetuximab has improved locoregional control and reduced mortality [3], although cisplatin-based chemoradiotherapy remains the standard of care until equivalence with radiotherapy plus cetuximab is reproducibly demonstrated [4].

Immunotherapy with biological response modifiers (BRMs) including OK-432 (picibanil) and BCG has been used to enhance antitumor immunity and biological responses have been reported; however, their effects on tumor immunity have been non-specific [5]. Tumor antigen-specific vaccinations have since been perceived as a potentially effective approach to improve outcomes by mobilizing antitumor immunity and reversing immune escape in cancer patients. Phase 1 studies using survivin-derived peptides and p53 peptide vaccines for patients with head and neck cancer were found to be safe and achieved moderate clinical outcomes [6,7]. On the other hand, efforts to restore latent antitumor immunity have focused on antibody-based interventions targeting cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) on T cells and its principal ligand (PD-L1) on tumor cells. The immune checkpoint inhibitor to either PD-1 or PD-L1 has produced significant antitumor activity with markedly less toxicity than the CTLA-4 inhibitor. In clinical trials on these immune checkpoint inhibitors, positive responses were observed in patients with melanoma, renal cancer, lung cancer, bladder cancer, and head and neck cancer [8,9].

Another recent advance in cancer treatments is the use of viruses that destroy tumors, *i.e.*, oncolytic viruses. Since some viruses exhibit oncolytic abilities, attempts to use

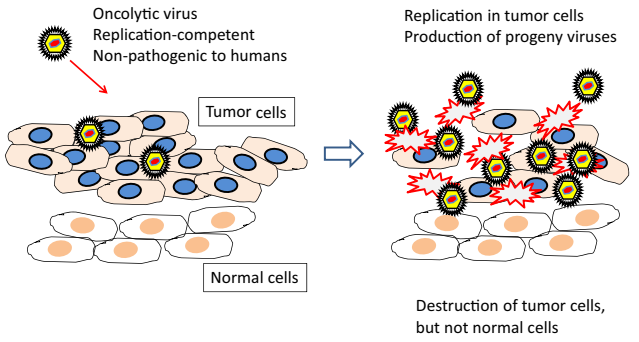

Figure 1 Schematic representation of selective killing effects of oncolytic viruses on tumor cells.

viruses as antitumor agents were made in the 1950s [10]. In Japan, the mumps virus was used and clinical effects were observed in 37 out of 90 terminal cancer patients; however, further clinical studies were not performed [11]. On the other hand, a virus is the most reliable vector to transfer human genes into deficient individuals. In 1990, a clinical trial on gene therapy was initiated to transfer the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency using a retrovirus to deliver the gene [12]. Gene therapy was also applied to the treatment of cancer. For example, the wild-type tumor suppressor gene p53 carrying a retrovirus or adenovirus was introduced into lung cancer patients in whom the p53 gene was mutated in order to restore the function of wild-type p53 [13,14]. Repeated intratumoral injections of adenovirus (Ad-p53) were tolerated well, resulted in the transgene expression of wild-type p53, and appeared to mediate antitumor activity in a subset of patients with advanced lung cancer. Viruses for gene therapy are generally replication-restricted in order to prevent the endless spread of viral infection in the body. A virus infects appropriate target cells, introduces genes into the cells, and its replication is then terminated. Target cells that acquire a tumor suppression gene are destroyed by the action of the gene, whereas cells devoid of the infection survive, and its effect on cancer is gradually lost, resulting in the failure of this therapy. This is a limitation of replication-restricted viruses. As a next step, replication-competent viruses were reconsidered as a tool to destroy a larger number of tumor cells by inoculated viruses and progeny viruses (Fig. 1). This concept was proposed by Martuza, a doctor of neurosurgery [15]. Mar-

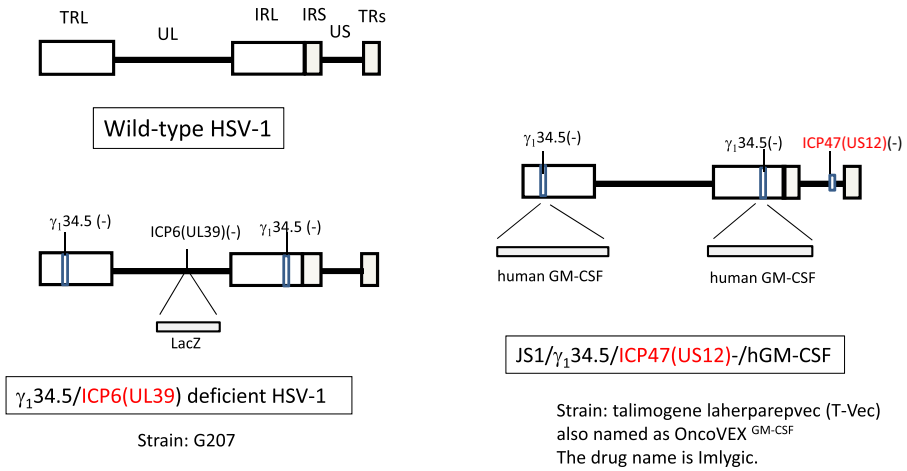

Figure 2 Schematic representation of the genomic structure of oncolytic HSV-1.
TRL, terminal repeat of the L component; UL, the L component; IRL, internal repeat of the L component; IRS, internal repeat of the S component; US, the S component; TRS, terminal repeat of the S component; GM-CSF, granulocyte macrophage colony-stimulating factor; ICP, infected cell protein.

tuza et al. attempted to destroy tumors in a glioma model using herpes simplex virus type 1 (HSV-1) in which the thymidine kinase (TK) gene was deleted by genetic engineering. This is the first published study to treat tumors with a modified replication-competent virus and the method was later called oncolytic virotherapy. HSV-1 frequently infects the oral mucosa and produces the primary infectious disease, gingivostomatitis, and recurrent disease, labial herpes. HSV-1 is one of the most extensively investigated viruses used for cancer therapy [16—20]. In this review, the development of viruses for use as a treatment of head and neck cancer including oral cancer is discussed with a focus on oncolytic HSV-1.

2. Development of an oncolytic virus

2.1. Genetic engineering of HSV-1

Since Martuza et al. published their work indicating that an engineered HSV-1 is applicable to the treatment of brain tumors, a number of viruses were considered to be oncolytc viruses for solid tumors. The major oncolytic DNA viruses are HSV, adenovirus, and vaccinia virus, and RNA viruses are coxsackie virus, reovirus, measles virus, Newcastle disease virus (NDV), and vesicular stomatitis virus (VSV) [16—23]. Oncolytic viruses may infect and replicate in tumor cells to produce progeny viruses, which is followed by the destruction of host tumor cells. If the progeny virus infects inherently susceptible cells and induces infectious disease, the treatment is not acceptable because the spread of infection must be limited to tumors. The initial HSV-1 vector tested was a TK gene-deficient mutant of HSV-1 [15]. However, the TK gene coded by the L component (UL) 23 of the HSV-1 genome is essential for the selective effects of the anti-herpes virus drug, aciclovir [24]; therefore, viral genes other than TK must be modified in subsequently developed oncolytic HSV-1s. Since the $\gamma_1$34.5 gene was found to be responsible for the neurovirulence of HSV-1 [25], HSV-1 vectors that were deficient in the $\gamma_1$34.5 gene were constructed. They were R3616, R489, HSV1716, NV1020, and G207 derived from wild-type HSV-1 strain F [26] (Fig. 2). When the $\gamma_1$34.5 gene was deleted, the ability of the virus to induce encephalitis was lost, even if the virus was directly injected into the brain. Moreover, the $\gamma_1$34.5 gene has been shown to play a major role in the selective targeting of tumor cells in HSV-1-mediated virotherapy. An infection with HSV-1 induces an interferon reaction, which activates double-stranded RNA-dependent protein kinase (PKR). PKR phosphorylates eukaryotic initiation factor 2 α (eIF-2α), which is essential for the translation of mRNA, resulting in the total shutdown of protein synthesis. The HSV-1 $\gamma_1$34.5 gene acts as a phosphatase accessory factor that recruits protein phosphatase 1α in order to dephosphorylate eIF-2α. As a consequence, protein synthesis continues unimpeded in cells in which HSV-1 expresses the $\gamma_1$34.5 gene. Thus, if the $\gamma_1$34.5 gene is deleted, the virus does not replicate in normal cells in which PKR is activated after infection by the virus. However, it was found that this mutant had the ability to replicate in tumor cells. The mechanisms responsible are not yet clear; however, RAS signals and down-stream mitogen-activated protein kinase (MAPK) kinase (MEK) may be activated in tumor cells constitutionally. These activated kinases block the activation of PKR by the HSV-1 infection such that the $\gamma_1$34.5 gene-deficient virus replicates in tumor cells, but not normal cells [27,28]. The efficiency of $\gamma_1$34.5 gene-deficient HSV-1 against tumors was demonstrated in basic and clinical studies without the induction of encephalitis [26,29—31]. Furthermore, another gene that encodes ribonucleotide reductase (RR) was modified to improve the specificity of viruses against tumor cells. RR is essential for viral replication and is provided by the host cell or virus itself; therefore, RR-dependent HSV-1 is permitted to replicate in proliferating tumor cells with RR activity only at a high level. HSV-1 with double mutations in the $\gamma_1$34.5 gene and UL39 gene encoding infected cell protein (ICP) 6 (RR) was subsequently constructed to decrease adverse effects; however, the killing effect of the virus was partly impaired [32]. It exerted cytotoxic effects on tumor

Table 1 Oncolytic HSV-1 investigated for its oncolytic ability to head and neck cancer.

Name	Modification	Clinical trial (HNSCC)	References
R849	$\gamma_1$34.5 deleted, with LacZ		[39,64]
HSV1716	$\gamma_1$34.5 deleted	Phase I	[49]
NV1020	One copy of $\gamma_1$34.5 deleted HSV-1/HSV-2 intertypic recombinant		[31]
G207	$\gamma_1$34.5 and ICP6 (UL39) deleted		[33]
G47 delta	$\gamma_1$34.5, ICP6 (UL39), and ICP47 (US12) deleted		[44]
Talimogene laherparepvec (T-Vec); OncoVEX^{GM-CSF} Imlygic (drug name)	$\gamma_1$34.5 and ICP47 (US12) deleted, with human GM-CSF	Phase I/II	[52]
OncoVEX $^{GALV/CD}$	$\gamma_1$34.5 and ICP47 (US12) deleted, with the GALV protein and CD		[35]
HF10	Mutations (glycoproteins, UL56)	Phase I	[54]
RH2	Mutations (gB, gD), $\gamma_1$34.5 deleted R849/HF recombinant		[40,41]

HNSCC, head and neck squamous cell carcinoma; GM-CSF, granulocyte macrophage colony-stimulating factor; GALV, gibbon ape leukemia virus; CD, cytosine deaminase.

cells and spared the normal mucosa when injected into the tumors of patients with head and neck cancer [33] (Table 1).

2.2. Fusogenic HSV-1

Several viruses have been shown to destroy their target cells through multinucleated syncytial formation, a process involving membrane fusion between infected and un-infected cells. The viral components that contribute to syncytial formation are mainly fusogenic membrane glycoproteins (FMGs). In order to efficiently spread the viral infection around the surrounding tumor cells, the FMG gene from gibbon ape leukemia virus (GALV) was inserted into the HSV-1 genome. The virus caused strong cell membrane fusion and significantly increased the potency of viral oncolysis [34]. HSV-1 expressing cytosine deaminase (CD) and FMG was reported to exert its lytic effects on head and neck SCC cells at a relatively low viral dose, whereas prodrug conversion by CD did not necessarily enhance therapy at doses that caused efficient cytotoxicity [35]. HF10, a clone of the spontaneously occurring and highly attenuated laboratory strain HF, induced extensive cell fusion and formed syncytia composed of large multinucleate cells [36]. HF10 was less virulent than wild-type HSV-1 in mice and did not induce encephalitis when injected into a peripheral site in mice; however, a high-dose intracerebral injection induced encephalitis. The genome of HF10 has large deletions at both ends of the L-region, an extensive genomic rearrangement, and it lacks the expression of UL56 and latency-associated transcript (LAT). The glycoprotein mutations responsible for cell fusion and a UL56 deletion have been implicated in its low neurovirulence [37]. Since no foreign genes were introduced into the genome, HF10 has an advantage over viruses with genetic engineering in that permission has been obtained from the Japanese government for clinical studies [38]. A recombinant between R849, a $\gamma_1$34.5 gene-deficient HSV-1 in which the LacZ gene was inserted, and HF, the parental virus of HF10, was subsequently produced [39,40]. This recombinant HSV-1 RH2 is deficient in the $\gamma_1$34.5 gene and has more mutations than wild-type viruses; the full sequences of the viral genome have been published [41]. Variations in the genes glycoprotein B (gB) and glycoprotein D (gD) are considered to be responsible for the fusogenic property of this virus. RH2 replicates efficiently, produces large syncytia, and destroys human oral SCC cells [40]. It was also found to suppress the growth of oral SCC xenografts in nude mice without any pathological features in animals.

3. Antitumor immunity

Cancer immunotherapy has wide appeal because of its hypothetical ability to eradicate residual or metastatic tumors that are difficult to manage by conventional treatments. The generation of whole tumor vaccines through tumor destruction *in vivo* has the potential to release the entire reservoir of tumor antigens in their native forms and configurations. In addition to the tumorcidal effects of viruses, oncolytic virus-mediated tumor destruction may provide an *in situ* source of tumor antigens to stimulate antitumor immunity. This specific feature of oncolytic viruses that is responsible for tumor immunity was reported in the 1990s. An intratumoral injection of oncolytic HSV-1 into murine colorectal carcinoma or neuroblastoma induced a tumor-specific immune response in synergistic mice that subsequently contributed to a significant reduction in the size of contralateral non-inoculated tumors or established tumors in the brain [42]. A previous study reported that fusogenic HSV-1 Synco-2D directly induced the cytolysis of tumor cells by syncytial formation and was considered to induce strong antitumor immunity, because virus-uninjected local and metastatic lung tumors

were markedly smaller than those in control mice [43]. A novel oncolytic HSV-1 was subsequently produced in which the $\gamma_1$34.5 gene was deleted to provide selective tumor cell proliferation, whereas the ICP 47 encoded by the S component (US)12 gene of the HSV-1 genome was deleted in order to enhance the antitumor response by antigen presentation and tumor cell killing through the up-regulation of US11, which occurs following this mutation. G47 delta is a mutant that contains three mutations, *i.e.*, $\gamma_1$34.5, ICP6, and ICP47. Its antitumor effects on human nasopharyngeal carcinoma have been demonstrated *in vitro* and *in vivo* [44]. Furthermore, the gene of human granulocyte macrophage colony-stimulating factor (GM-CSF) was inserted into the HSV-1 vector backbone with the deletions of the $\gamma_1$34.5 and ICP47 (US12) genes [45]. GM-CSF is a potent immune stimulator that promotes the differentiation of progenitor cells into dendritic cells. The combination of GM-CSF with oncolytic therapy may be particularly effective because cell death accompanying virus replication serves to release tumor antigens efficiently, which is required for GM-CSF-enhanced immune responses. The HSV-1 vector with GM-CSF was initially named OncoVEX$^{GM\text{-}CSF}$ and was changed to talimogene laherparepvec (T-Vec); its drug name is Imlygic (Fig. 2). The treatment of disseminated peritoneal tumors with HF10 has been shown to induce specific antitumor immune responses [36]. The enhancing effects of R849/HF recombinant RH2 on tumor immunity were also demonstrated using a synergic mouse model. Two tumors were produced on the back of the C3H mouse and RH2 was injected into one tumor while the contralateral tumor was left untreated. Although the suppression of RH2-injected tumors was significantly greater than that of uninjected tumors in the treated group, the suppression of contralateral tumors was also greater in the treated group than in the untreated group. Immune cell infiltration, particularly, CD8$^+$T cells played a major role in this immune response [46]. Furthermore, cell death with immunogenic potential, *i.e.*, immunogenic cell death, was also demonstrated in RH2-infected SCC cells [47].

4. Clinical study

4.1. Adverse effects

Since viruses have been shown to effectively reduce tumor volumes *in vivo*, clinical trials have been undertaken using adenovirus, HSV-1, coxsackie virus, reovirus, HSV, vaccinia virus, and VSV. Adenovirus, reovirus, and HSV have been used in clinical studies for head and neck cancer [16,20,21,23,48,49]. In a phase 1 study with HSV-1 G207 for recurrent malignant glioma, side effects were limited to mild to moderate febrile reactions and localized erythematous/inflammatory reactions at the injected sites of tumors [50]. While commonly referred to as influenza-like symptoms, side effects are generally milder, but may be more robust in patients who were seronegative for the parental viral species at their first dose or when a high viral dose is given by an intravenous route [23]. Safety issues with oncolytic agents are mostly the same as those for live attenuated viral vaccines, and this is the general direction toward which regulatory requirements are moving, albeit with considerable country-to-country variations.

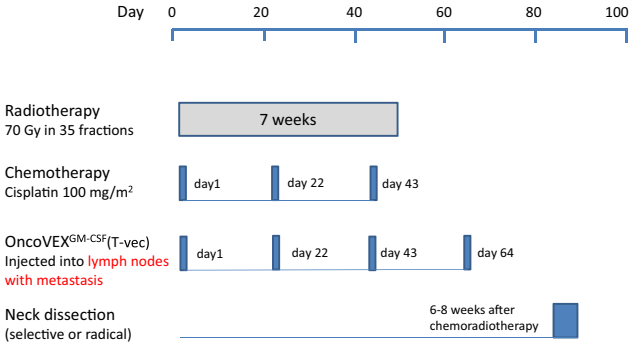

Figure 3 Treatment schedule of cisplatin-based chemoradiotherapy with oncolytic HSV-1 as reported by Harrington et al. [52].

4.2. HSV-1 talimogene laherparepvec (T-Vec)

While case reports have described significant responses to oncolytic viruses in patients with disseminated diseases, only a small number of phase II/III trials have been published to date [51,52]. The most recent of these and the first trail on oncolytic therapy to show its tangible benefits is the phase III trial on HSV-1 T-Vec [53]. In this trial, patients with treated/untreated unresectable stage IIIB/C or stage IV melanoma were randomized 2:1 to receive intratumoral T-Vec or subcutaneous recombinant GM-CSF. Among 436 patients, the durable responsible rate was significantly higher with T-Vec (16.3%) than with GM-CSF (2.1%). The overall response rate was also higher in the T-Vec arm (26.4%) than in the GM-CSF arm (5.7%). Median overall survival (OS) was 23.3 months with T-Vec and 18.9 months with GM-CSF ($P = 0.051$). The most common adverse events with T-Vec were fatigue, chills, and pyrexia. T-Vec was effective, particularly in untreated patients or those with stage IIIB, IIIC, or IV1a disease. The clinical effects of T-Vec were also investigated in combination with radiotherapy and cisplatin in phase I/II study for patients with head and neck cancer in untreated stage III/IV [52] (Fig. 3). Seventeen patients were enrolled. Patients received chemoradiotherapy (70 Gy in 35 fractions with concomitant cisplatin 100 mg/m^2 on days 1, 22, and 43) and dose-escalating T-Vec by an intratumoral injection on days 1, 22, 43, and 64. In this study, the intratumoral injection means an injection of T-Vec into metastatic lymph nodes. Patients underwent neck dissection 6—8 weeks after the end of chemoradiotherapy. Locoregional control was achieved in all patients, with a 76.5% relapse-free rate. Disease-specific survival was 82.4% at a median follow-up of 29 weeks (range, 19—40 months).

4.3. HSV-1 HF10

HF10 was demonstrated to be effective as an oncolytic agent using various murine tumor models, including melanoma, metastatic breast cancer, bladder cancer, ovarian cancer, sarcoma, and colorectal cancer [18]. An intratumoral injection of HF10 was performed in a small number of Japanese patients with recurrent breast cancer and head and neck cancer without significant adverse effects. Metastatic skin nodules (lymph node metastasis) of head and neck cancer

were injected with HF10 once a day for 3 days. HF10 replicated in the tumors, spread well in the tumor nodules, and caused cell death in a large population of tumor cells [54]. In a phase 1 clinical trial for unresectable pancreatic cancer, HF10 was injected intratumorally 3 times and exhibited some therapeutic potential. Tumor responses were classified as stable disease in 3 patients, a partial response in 1 patient, and progressive disease in 2 patients [55].

5. New combination therapy

5.1. Oncolytic virus and immune checkpoint inhibitors

Antibodies targeting CTLA-4 and PD-1/PDL-1 are already used clinically for a range of malignancies, including melanoma, and have had very positive effects. Melanoma antigen-specific vaccines have been evaluated in early phase clinical trials in adjuvant settings and were found to increase the expression of PD-1 at the injection site. Similarly, there is emerging evidence to show that oncolytic viruses regulate the expression of PD1/PDL-1 in cancer cells, thereby providing a strong rational for combination therapy with immune checkpoint inhibitors. The combination of anti-PD-1 and reovirus led to longer survival than that with either agent alone [56]. An anti-PD-1 antibody treatment down-regulated regulatory T cell activity and increased effectiveness of NK-cell-mediated lysis in malignant cells infected with reovirus. In a phase I trial, the anti-CTLA-4 monoclonal antibody, ipilimumab, was combined with a poxvirus-based vaccine in patients with castrate-resistant prostate cancer [57]. The side effect profile did not appear to differ significantly over single agent therapy with ipilimumab. Most recently, encouraging findings have been reported from combination T-Vec with an anti-CTLA-4 antibody [58]. Ongoing T-Vec studies include evaluations in the safety and efficacy of T-Vec plus ipilimumab *versus* ipilimumab alone and T-Vec plus pembrolizumab (anti-PD-1) *versus* pembrolizumab alone [23].

5.2. A specific reagent to enhance the replication of oncolytic HSV-1

Certain agents have been shown to induce the differentiation of malignant cells [59]. Tumor cells committed to differentiate lose their ability to proliferate and propagate when transplanted into animals. Hexamethylene bisacetamide (HMBA), a compound structurally related to dimethyl sulfoxide, was found to induce the differentiation of erythroleukemia cells [60] and has been tested in patients with refractory solid tumors [61]. HMBA is of interest because it enhances the replication of wild-type HSV-1 in SCC cells and the addition of the compound facilitated the reactivation of HSV-1 or HSV-2 in animal models [62,63]. The replication of $\gamma_1$34.5 gene-deficient HSV-1 R849 in oral SCC cells was also enhanced in the presence of HMBA in oral SCC cells. In an animal study, the growth of nude mouse tumors was markedly suppressed by R849 in combination with HMBA and the survival of co-treated animals was significantly longer than that of animals treated with R849 only. HMBA enhances the antitumor activity of an inoculated virus through the expression of immediate early genes without increasing its toxicity [64]. It may be useful as an enhancing agent for oncolytic therapy with HSV-1 in cancer patients.

6. Conclusion

In oncolytic virotherapy, there are many hurdles that must still be overcome. They include selective virus replication in tumor cells, efficient virus delivery and intratumoral spread, stimulation of antitumor immunity, immune-mediated clearance of virion particles from patient body, limited ability of pre-clinical tumor model systems to actually predict the efficacy, and environmental viral shedding. Nevertheless, the mechanisms by which oncolytic HSV-1 exerts its antitumor effects are completely different from those of conventional and recent therapies with chemotherapeutic agents, radiation, peptide vaccines, and monoclonal antibodies targeting EGFR, CTLA-4, and PD-1/PD-L1; it induces cell lysis by viral replication in the tumor and releases tumor antigens to potentiate tumor immunity. In phase III clinical trials, oncolytic HSV-1 showed specific antitumor immunity and clinical responses in a significant fraction of melanoma patients. The synergistic and/or additive effects of oncolytic viruses have been demonstrated in combination with conventional modes of therapy. Based on encouraging evidence supporting the safety and potential efficacy of oncolytic viruses, there is a significant chance that viruses may become an entirely new modality for the treatment of oral cancer.

Conflict of interest

The authors declare that no conflicts of interest exist.

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