Feasibility of herpes simplex virus type 1 mutants labeled with radionuclides for tumor treatment

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Abstract

For over one hundred years, viruses have been recognized as capable of killing tumor cells. At present, people are still researching and constructing more suitable oncolytic viruses for treating different malignant tumors. Although extensive studies have demonstrated that herpes simplex virus type 1 (HSV-1) is the most potential oncolytic virus, therapies based on herpes simplex virus type 1 vectors still arouse bio-safety and risk management issues. Researchers have therefore introduced the new idea of treating cancer with HSV-1 mutants labeled with radionuclides, combining radionuclide and oncolytic virus therapies. This overview briefly summarizes the status and mechanisms by which oncolytic viruses kill tumor cells, discusses the application of HSV-1 and HSV-1 derived vectors for tumor therapy, and demonstrates the feasibility and prospect of HSV-1 mutants labeled with radionuclides for treating tumors.

Key words: Oncolytic virus; Herpes simplex virus type 1; Mutant; Radionuclide; Tumor therapy

INTRODUCTION

Oncolytic virus[1], a tumor-specific replicating virus, is one of a number of natural or engineered viruses that can selectively replicate in and cleave tumor cells, but not kill normal somatic cells. There are six major mechanisms by which viruses can lyse tumor cells: (1) Viruses can replicate, package in and cleave tumor cells; (2) Similar to non structure protein (NS protein), some protein particles produced by virus replication can induce host cell apoptosis; (3) Both nonspecific and specific immune responses as well as immunoreactive sensitivity to cytokine (TNF-α, IFN-γ) associated with virus infection can accelerate tumor cell lysis; (4) Virus associated antigen can enhance MHC- I expression on tumor cells, and increase the ability of antigenic specific cytotoxic T cells to kill tumor cells; (5) Oncolytic virus can enhance the sensitivity of tumor cells to radiotherapy and chemotherapy; and (6) Oncolytic virus can carry and express cancer therapeutic genes.

At present, many engineered oncolytic viruses have been used in animal or human studies. Some of them have been or are ongoing into phase Ⅲ clinical trials, especially adenovirus. ONYX-015[2,3], a derivative of adenovirus with a deletion of the E1B-55KD gene, can kill p53-deficient tumor cells, but not cells with functional p53. Many limitations were found, however, in its phase Ⅱ clinical trial; ONYX-15 could neither treat tumors sufficiently, infect secondary tumor metastases efficiently, nor spread sufficiently in solid tumors[2,3]. Nevertheless, evident organ impairment, toxicity and environmental events have not been found.

Several other oncolytic viruses (Newcastle disease virus, reovirus, retrovirus, rabies virus) have not been extensively used in research and clinical therapy, due to small genome size, limited host range, unclear replication mechanisms, *etc*.

RESEARCH AND CLINICAL APPLICATIONS OF HERPES SIMPLEX VIRUS TYPE 1 FOR TUMOR TREATMENT

Compared with other viruses, herpes simplex virus type 1 (HSV-1) has a number of favorable properties for cancer treatment[4]. (1) In spite of its neurotropism, HSV-1 possesses a wide range of host cells, which is superior to adenovirus. (2) HSV-1 is able to infect dividing cells as well as nondividing cells. (3) Its DNA has been completely sequenced[5], and the genome of 152 kb has exceeded adenovirus genome of 36 kb. Furthermore, transgenes can replace as much as 30 kb of the deleted HSV genome in replication-defective HSV-1 mutants, and

simultaneous delivery of multiple transgene and insertion of heterogonous promoters are allowed. (4) Its genome will not integrate into the cellular genome, resulting in little insertional mutagenesis, which is the major concern in retrovirus and adeno-associated virus vectors. (5) The entire replication cycle is usually about 20 h in permissive cells[5], and that of adenovirus is 48-72 h[6]. (6) Recombinant HSV-1 is easily engineered, and purified viruses with a high titer can be routinely prepared. (7) HSV-1 can efficiently infect different cells[7], which is advantageous for the generalization from preclinical results to clinical trials. In contrast, the application of adenovirus vectors has been limited by few animal models. (8) HSV-1 infection is able to spread through both cell junctions and extracellular spaces and can penetrate into solid tumors with less systemic spread. (9) HSV-1 rarely causes life-threatening illness even in immune-competent adults. (10) HSV-1 infection can be treated using several anti-HSV-1 drugs (such as Acyclovir and Famciclovir)[8].

Two main types of HSV-1 vectors are used in tumor therapy: replication-defective vectors and conditionally replication vectors. They can either terminate viral lytic infection or only confine themselves in certain types of cells.

Replication-defective vectors, in which either one of a few essential viral genes are deleted and then transgene expression cassettes are inserted in a viral genome, can effectively express transgene products, but are unable to replicate in cells unless host cells can supply the deleted viral functions. The HSV-1 mutant is used as a transgene in nerve cells. The HSV-1 thymidine kinase (TK) gene, a suicide gene encoded by U_L23, is unique and most frequently used. TK protein can transform innocuous prodrugs such as GCV into cytotoxic drugs, resulting in termination of DNA synthesis in active cells, especially in tumor cells. Furthermore, bystander effects generated by TK/GCV systems are also toxic to tumor cells, but not towards neurons and quiescent glia[9]. Besides the TK gene[10], other cancer therapeutic genes such as connexin-43[11], p53[12], IL-2[13], granulocyte-macrophage colony-stimulating factor (GM-CSF)[13], IL-12[14] and interferon (IFN-γ)[15], have also been delivered in the replication-defective HSV-1 vectors for treating malignant gliomas and other types of tumors, and an antitumor effect has been demonstrated by intratumoral injection in animal models. In addition, multiple therapeutic genes, for example, co-expressing TK and TNF-α[16], TK, connexin-43 and TNF-α[17], TK and IL-12[18] , have simultaneously been delivered in replication-defective HSV-1 vectors in extensive studies. As a result, tumor growth was significantly inhibited.

Conditionally replicating vectors, generated by deleting some nonessential viral genes, can preferentially infect, replicate in, and lyse tumor cells. Therapeutic transgenes can also augment the antitumor effect.

Recently, there have been many reports about HSV-1 mutants. In several animal tumor models, HSV-1 mutants with TK gene deletion were created and tested for oncolytic virotherapy, which could induce tumor regression following intra-neoplastic administration[19]. The U_L39-deleted virus, hrR3, efficiently replicates in malignant cells but proliferates less in normal cells[20]. It was demonstrated that hrR3 had produced significant antitumor effects and survival benefits in animal models such for brain, colon, pancreas and liver cancers. In animal studies, HSV1716[21], R3616[22,23] and R4009[22,23], which are the ICP34.5-deleted strains, are able to replicate when inoculated into neuron and other non-replicating cells. In contrast, in animal models of glioma, mesothelioma, melanoma, ovarian and lung cancer, HSV1716, R3616 and R4009 are replicated significantly. G207[24,25], containing deletions of both copies of ICP34.5 gene and a insertion of *E. coli* lacZ gene in U_L39 gene which encodes ICP6, had some favorable properties for cancer treatment. Competent replication in tumor cells, attenuated neurovirulence, GCV hypersensitivity, temperature sensitivity and the product of lacZ gene were easily detectable. G47Δ[26], a derivative of G207 with an additional ICP47 gene deletion, could more efficaciously inhibit tumor growth *in vivo* than its parent G207, while safety was unaffected[26]. Under the name of NV1020[27], R7020[27,28] contained a 15kb deletion of ICP34.5, the deletions of U_L24, U_L55, U_L56 and endogenous TK gene and the insertion of exogenous TK gene which was controlled by $\alpha4$ promotor. Compared with R3616[22,23] (double ICP34.5-deletion mutant), R7020 was more efficacious in inhibiting the growth of tumors, and more sensitive to ACV and GCV. Myb34.5[29] had the deletion of both endogenous copies of the ICP34.5 gene and re-insertion of this gene into the ICP6 locus, with the new ICP34.5 gene under the control of the B-myb promoter. In contrast to hrR3, Myb34.5 was active in tumor cells, but was more attenuated in normal cells.

Although the usefulness of HSV-1 vectors for treating tumors have been confirmed, there are some limitations in their application. (1) It is more difficult to produce multiple gene deleted HSV-1 vectors than the wild-type virus, resulting in a lower yield. The opportunity of homologous recombination of wild-type HSV-1 with recombinant viral mutants is concerned. (2) It is particularly difficult to keep long-term stability (over 6-mo period) of HSV-1 derived vectors, either in aqueous solution or in lyophilized form. (3) The virus may rapidly spread in individuals with immunodeficiency. The efficacy may be reduced by immune response induced by antiviral or antitransgene products. (4) Attention must be paid to the safety in use of HSV-1 because HSV-1, a human pathogen, has broad cell type tropism and high replication capacity. So the safety and efficacy of HSV-1 mutants for tumor treatment are still a focus of future studies.

SUPERIORITY AND PROSPECT OF HSV-1 MUTANTS LABELED WITH RADIONUCLIDES FOR TUMOR TREATMENT

HSV-1 mutants can selectively infect tumor cells, but not suppress the growth of normal cells. There may be a synergistic antitumor effect, when they are labeled with radionuclide. With the help of the viral vectors, radionuclide will be carried into tumor tissues and bring

about radiation damage to tumor cells. It is rarely reported whether HSV-1 can be labeled with radionuclide, whether viral bioactivity may be influenced by the virus labeled or radionuclide which will enter into tumor cells together with virus. We analyzed these issues in this overview.

HSV-1 has an enveloped double-stranded nucleic acid (dsDNA). Located in the core of the virus, dsDNA is surrounded by a protein shell called a capsid which consists of 162 capsomeres arranged in a T = 16 icosahedral symmetry. The channels which are controlled by tegument proteins are contained in the capsid, controlling the transport of dsDNA through the channel. Surrounding the capsid, an amorphous tegument contains at least eight types of proteins that play an important role during HSV-1 infection. An outer envelope is composed of lipid bilayer with about 13 different viral glycoproteins.

It is well known that the HSV-1 envelope and tegument-capsid surrounding dsDNA consist of multiple proteins, about 60%-80% protein of the whole structure elements. However, HSV-1 mutants are generally constructed by modifying the dsDNA without altering their tegument-capsid. There are enough proteins, which well fit the labeling. Furthermore, the proteins such as monoclonal antibody, polypeptide and ligand, can all be labeled successfully[30,31]. As for radionuclide, there are ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{99m}Tc, ^{188}Re, ^{186}Re, ^{90}Y and so on. In a word, the proteins contained in HSV-1 structural elements are considerably suitable for radiolabelling.

During initial infection, HSV-1 attaches first to the host cell surface receptor, and fuses with the membrane of host cells, and the de-enveloped tegument-capsid is efficiently and rapidly transported to the nuclear pore complexes, where the viral dsDNA is released. The capsid with associated tegument structures can also be carried to the nuclear pore through the microtubules[32]. The transition from virtual attachment to penetration is very rapid and takes only several minutes[33]. At the early phase of virus infection, the viral envelope glycoproteins, gC and gB, bind to cell surface heparin sulfate[34]. Then, viral glycoprotein gD binds to certain cell surface receptor (e.g., nectin-1a, nectin-1b, 2a, 2d, HveA), contributing to virion-cell fusion[35]. Besides, three other viral envelope glycoproteins, gB, gH and gL, have been shown to be helpful for the HSV-1 penetration into host cells[36]. As a result, the viral proteins are delivered into different metastructures. So, it is feasible that HSV-1 vector mutants could carry radionuclide-labeled proteins into tumor cells.

Could radiation from radiolabeling of viruses destroy their structures and damage their biologic characteristics? A study[37] on the combination of gene therapy with radiotherapy for tumors revealed that the gene products in mice lung cancer cells had increased dose-dependently, when irradiated by gamma-ray at doses of 2-40 Gy followed by AdCMVlu transfection. The efficacy could be up to 24 times higher, and tumor growth was inhibited. Kanazawa[38] demonstrated that the number of replicative dsDNA of the laryngeal cancer cell line (HEp-2) and Henrietta Lacks (HeLa) strain of cancer cells had a significant enhancement in the combination of virus system and radiotherapy. The virus system was composed of adeno-associated virus (AAV) vectors,

the HSV-1 thymidine kinase (HSV-tk) and ganciclovir (GCV). The radiation dosage was 4Gy. These experiments *in vitro* suggested that the combination of AAVtk/GCV system with radiotherapy was significantly effective in the treatment of cancers. Weichselbaum RR[39] found radiation could induce the transcription of CarG elements in the Egr-1 promoter sequences which lies the upstream of TNF-α cDNA. At the same time, TNF-α obtained a high expression. Generally, radiotherapy would stimulate the virus replication, resulting in a higher HSV-1 antitumor activity[40]. Other studies had also indicated that ionizing radiation could enhance anti-cancer activity of hrR3 without altering its replication[40]. Similarly, a synergistic antitumor response, with more tumor regression and better survival rate, was induced by the combination of ionizing radiation and R3616 deleted ICP34.5[41]. So, radionuclide labeled to HSV-1 mutants does not damage the viral biological function, but can enhance the antitumor effect.

It is extremely important that compounds labeled with radionuclide should possess a good stability *in vivo*. The labeled compounds should stay in the tumor cell cytoplasm while the HSV-1 mutant dsDNA enters the nucleus. HSV-1 recombinant-labeled radionuclide should, therefore, have a favourable stability before penetration into the tumor cell nucleus. Of course, suitable radiolabeling methods which can keep labeled compounds stable should be well considered. Diethylenetriaminepentaacetic acid (DTPA) is known as one of the difunctional chelators. This kind of chelator has a stereochemical structure group which is found to tightly hold radionuclide and amino acids fragments active group by a covalent link. Thus, radionuclide (^{188}Re, ^{90}Y) is indirectly linked up with protein molecule, and the compounds labeled with radionuclide is stable *in vivo*. Moreover, the biochemical activity of radiolabeled complex will not be influenced. As a chelating agent, DTPA has a potential and broad applicability[30,31].

As far as radiolabeling and the structure of HSV-1 are concerned, the feasibility of labeling radionuclide to HSV-1 mutants is demonstrated by a plenty of animal experiments and clinical trials. Furthermore, once the radionuclide is introduce, nuclear medicine approaches may be employed in the clinic to monitor and assess the therapeutic efficacy *in vitro* in a non-invasive manner.

In order to increase the efficiency of HSV-1 mutants in killing tumor cells, we intend to construct HSV-1 GRT, by deleting ICP 47 gene and one copy of ICP 34.5 gene and inserting *E. coli* Z gene and TNFα gene in U$_L$39 gene. Then HSV-1 GRT can be labelled with ^{131}I or ^{188}Re directly or indirectly. We will study the expression of HSV-1 GRT or radioactive compounds (^{131}I-HSV-1 GRT, ^{188}Re-HSV-1 GRT and ^{188}Re-DTPA-HSV-1 GRT) in tumor cells and mechanism of inhibiting or killing tumor cells. In other words, our aim is to develop an ideal drug which can combine viral oncolysis with radionuclids therapy. With the help of the HSV-1 GRT, radionuclids (^{131}I or ^{188}Re) can be carried into tumor tissues and bring about radiation damage to tumor cells.

CONCLUSION

HSV-1 mutants provide a choice for oncolytic viruses

to selectively target and kill tumor cells, and they are also attractive vectors helping radionuclide get to focal tumor tissues. The combination of viral oncolysis with radionuclide therapy will achieve a synergistic anticancer effect, with more inhibition of tumor growth, less toxicity and fewer side effects than either HSV-1 mutants or radionuclide therapy. It is reasonably concluded that HSV-1 mutants labeled with radionuclide will be a potential focus for tumor treatment.

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