Review Article

Oncolytic Viruses in Head and Neck Cancer: A New Ray of Hope in the Management Protocol

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Abstract

This paper intends to highlight the different types of oncolytic viruses (OVs), mechanism of tumor specificity, its safety, and various obstacles in the design of treatment and combination therapy utilizing oncotherapy. Search was conducted using the internet-based search engines and scholarly bibliographic databases with key words such as OVs, head and neck cancer, viruses, oral squamous cell carcinoma, and gene therapy. Revolutionary technologies in the field of cancer treatment have gone through a series changes leading to the development of innovative therapeutic strategies. Oncolytic virotherapy is one such therapeutic approach that has awaited phase III clinical trial validation. OVs are self-replicating, tumor selective and lyse cancer cells following viral infection. By modifying the viral genome, it is possible to direct their toxicity toward cancer cells. Viruses that are used for treatment of head and neck cancer are either naturally occurring or genetically modified. OVs are tumor selective and potential anticancer agents. Virotherapy may become the standard of care and part of combination therapy in the management of head and neck cancer in the future.

Keywords: Gene therapy, Head and neck cancer, Oncolytic virus, Oral squamous cell cancer, Viruses

Introduction

Cancer is a major cause of death worldwide, in that head and neck cancer accounts for 5% of all the malignancies.[1,2] There has been tremendous improvement in the management of head and neck cancer including surgery, advanced chemotherapy, radiotherapy, immunotherapy, and gene therapy. All the treatment modalities currently employed are associated with potential adverse effects. Hence, there is an urgent need of a treatment modality that targets cancer cell and has minimal side-effects. One such upcoming approach is the use of viruses to kill cancer cells.[3-5]

Most of people are aware of viruses being pathogenic microorganism that infects cells and replicates in them using DNA of host cells and ultimately lyse and kill the cells.[6,7] On applying this old hypothesis, it was possible to harness this activity for therapeutic purposes by modifying viral genome to target their toxicity toward cancer cells.[8,9] Oncolytic virotherapy is an innovative approach that has awaited phase III clinical trial for evaluation.[10] Research on oncolytic viruses (OVs) began as early as 1950's, but it was only in 1991 that herpes simplex virus 1 (HSV-1) with deletion of thymidine kinase gene became first genetically engineered OVs to be tested in the laboratory.[11]

The objective of this review is to highlight the history of OVs, their mechanism of action, tumor specificity, and clinical trials in the head and neck cancer, safety and obstacles in the use of OVs.

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Methods of Literature Search

Various internet-based popular search engines (Google, Google Scholar), scholarly search bibliographic databases (PubMed, PubMed Central, Medline Plus, Medknow) and textbooks were searched from the year 1964 until the year 2012 based keywords such as "OV," "oral cancer," "head and neck cancer," "gene therapy," "viruses." The search was limited to articles published in English, and after examining the titles and abstracts of the articles, a total of 64 publications were finally considered to review the OVs.

What are oncolytic viruses?

Viruses that specifically infect and lyse tumor cell, sparing normal ones are termed as "OVs." The therapeutic approach which utilizes these OVs as agents to fight against cancer is termed as "oncolytic virotherapy".[3,12]

History of oncolytic viruses

Since their discovery, the viruses have occupied center stage and have generated considerable interest as possible agents for tumor regression. Early case reports emphasized that there was a brief period of clinical remission in patients suffering from cancer, when these patients acquired viral infection. This observation provided the basis for research on OVs. Employing these viruses for therapeutic purposes was first suggested at the beginning of the 20th century with hepatitis virus being the first to be used for the therapy, followed by Egypt 101 virus, adenovirus, picornavirus and mumps virus. At this time, clinical studies performed were quite alarming in the context of ethical standards, as the therapeutic material administered to cancer patients often consisted of infections body fluids or infected tissues harvested from patients suffering from viral infections. Most often, especially in immunocompromised patients the viral infection persisted although there was tumor regression and lead to the death of the patients. With the advent of rodent models, testing *in vivo* antitumor activity of OVs under controlled laboratory conditions was possible. Later some animal viruses which were nonpathogenic in humans, but capable of destroying human tumors were employed. However, this had a risk of virus evolution giving rise to a new human pathogen. In contrast, certain animal viruses like new castle disease virus found to be safe in humans formed a platform for the development of OVs. There was a drastic fall in the field of virotherapy during the period of 1950's and 1960's due to the limited success, until recently until the advent of genetic engineering. Viruses can be genetically modified to eliminate their pathogenicity and also target tumor specificity, thus producing a safer OV.[13-16]

Types of oncolytic viruses

Viruses may be nononcolytic nonreplicating viruses or OVs. Nononcolytic nonreplicating viruses are used as vectors for gene therapy.[17] OVs can be wildtype or naturally attenuated strains of viruses that possess an inherent property of selective replication and lysis of cancer cells or they can be genetically modified to selectively replicate and lyse cancer cells.[18] Naturally occurring OVs include reovirus, new castle disease virus, vesicular stomatitis virus, etc., These viruses have very low pathogenicity in humans (reovirus) and humans are not the natural hosts (new castle disease virus and vesicular stomatitis virus).[19,20] Genetically modified viruses have been engineered to be more tumor specific and less pathogenic to normal tissues.[21] Examples for this group include adenovirus, HSV and vaccinia virus.

Mechanism of action

Oncolytic viruses destroy malignant cells through a combination of different mechanism. One important mechanism is by direct cancer cell lysis secondary to viral replication. It causes direct cytotoxicity by producing the protein that is lytic to tumor cells. OVs induce specific and nonspecific immune response against the tumor. Oncolytic virotherapy also sensitizes tumor cells to chemotherapy and radiotherapy.[22]

Mechanism of tumor specificity

Tumor specificity of OVs are mediated by an array of different mechanisms. One such mechanism is due to the presence of defective antiviral defense mechanism in the tumor cells. In normal cells, viral protein synthesis is inhibited by activated interferon and protein kinase pathway (PKR). When virus enters the body, interferons are activated, which in turn activates PKR pathway leading to inhibition of viral protein synthesis. In cancer cells, interferons are inactivated, and Ras pathways are activated (Ras inactivates PKR pathway). This results in defective antiviral response in cancer cells. OVs that act by these mechanisms are some of the naturally found viruses such as new castle disease virus, reovirus, vaccinia virus, and vesicular stomatitis virus.[21,23,24]

Some of the tumor cells have specific receptor that are overexpressed or have mutated receptor that are unique to cancer cells, which are absent in normal cells. Through these the OV can enter tumor cells and replicate in them, thus killing them, sparing the normal ones.[25-27]

Tumor specificity can also be achieved by using tissue specific promoters. Virus is incorporated with gene promoters that are particularly active in specific tumors, and help virus target tumor cells.[28] For example, papilloma virus promoters show a high level of specificity to oral cancer cells. Therefore, when HSV-1 strain was incorporated with papilloma virus promoters, it facilitated the virus strain to aim the oral cancer cells.[29,30]

In addition, proteolytic processing of viruses within the tumor cells found to be an alternate mechanism through which virus targets the tumor cells. For instance, reovirus requires proteolytic breakdown of outer viral coat into subviral particles for the successful infection of the cells. Some of the tumors found to have excess of proteases in the tumor microenvironment facilitate virus activation in the tumor cells.[17,31,32]

Different oncolytic viruses and their clinical trials

Several types of viruses have been employed to date which can target cancer cells. These include adenovirus, new castle disease virus, pox virus, HSV, picornavirus, vesicular stomatitis virus, and reovirus. Both DNA and RNA viruses have been used in oncolytic virotherapy, but DNA viruses are more frequently used as these are more amenable to genetic manipulation.[16] Various studies have been conducted in evaluating the efficacy of adenovirus and HSV in the treatment of head and neck cancer [Table 1]. The world's first OV approved by China's State Food and Drug Administration in 2005, was a genetically modified adenovirus-H101.[11]

Table 1: Clinical trials in head and neck cancer

Author/ reference	Oncolytic virus and/or other agents	Dose	Response (%)	Adverse effects
[33]	dl1520 (Onyx-015; adenovirus)-intratumoral injection	5×10^{5}-5×10^{9} viral particles	3/22 (14) of response in patients with HNSCC	Nausea, chills, injection site pain
[34]	dl1520 (Onyx-015; adenovirus)-intratumoral injection	5×10^{8} viral particle	5/37 (14) response in patients with HNSCC	Injection site pain, asthenia, fever
[35]	dl1520 (Onyx-015; adenovirus)-intratumoral injection and chemotherapeutic agent cisplastin and 5-FU	5×10^{8} viral particle cisplatin 80 mg/m^2 5-FU 800-1000 mg/m^2	19/37 (53) response in patients with HNSCC	Asthenia, fever, chills
[36,37]	H101 - intratumoral infection; and chemotherapy (drugs and doses not available)	5×10^{11} viral particle and chemotherapeutic agent	14/50 (28) in patients with solid cancer	Fever, injection site pain, leukopenia, nausea, vomiting

HNSCC: Head and neck squamous cell carcinoma, 5-FU: 5-flurouracil

Adenovirus is a nonenveloped virus with a double-stranded DNA. It is the first engineered replication selective virus to be used in humans. Adenoviral vectors have deletions in viral genes that function to suppress the host immune response or have impaired cytolytic activity. Various oncolytic adenoviruses that have been attempted in the treatment of head and neck cancer are Onyx 015, H101, and KH 901). Onyx 015 (also known as *dl*1520, H101), is a replication selective adenovirus with deletions in E1B-55K region effective in cells with inactive p53.[38] H101 adenovirus was tested in patients with head and neck cancer by intratumoral injection and has been approved in china for human use.[11,39] KH901 adenovirus is engineered with human telomerase promoter and armed with granulocyte macrophage colony stimulating factor.[5,40]

Rudin *et al*. conducted a study to assess the feasibility and activity of ONYX-015 administered topically as a mouthwash in patients with clinically apparent premalignant oral dysplasia. Oral dysplastic lesions have a high prevalence of *p53* dysfunction, would be an ideal target for ONYX-015 therapy. Nineteen patients were administered with ONYX-015 mouthwash and observed that there was histologic resolution of dysplasia in 7 (37%) of 19 patients, and the grade of dysplasia improved in one additional patient. The responses were transient, but toxicity profile was favorable and they concluded that oncolytic virotherapy is a tolerable and feasible approach to cancer prevention.[41]

Herpes simplex virus is an enveloped, double stranded DNA virus with 152 kb long genome with three major gene regions are alpha, beta, and gamma.[42,43] Each of the genes act to regulate viral entry, replication and multiplication inside the nuclei of infected host cells.[44] Varieties of mutants have been tried using HSV-1 virus including functional inactivation of the viral genes that encode for thymidine kinase, ribonucleotide reductase and infected cell protein 34.5.[45] Two oncolytic HSVs that have been tested in the head and neck cancer patients are HSV HF10 and HSV-171. HSV HF10 which lacks the expression of UL56 has been administered in two patients with head and neck SCC with metastatic skin lesions.[46] Two patients received intratumoral administration of 105 pfu HF10, and the injected tumors were excised. The injected tumors showed greater infiltration of CD4-positive and CD8-positive cells than the uninjected tumors when examined 2 weeks postinjection.[47] Another oncolytic HSV-1716 (with deletion in g 34.5) is generally used for oncolytic virotherapy with HSV-1 because of its lack of neurovirulence.[48] HSV-1716 was administered intratumorally (1×10^5 or 5×10^5 pfu) into 20 patients with oral squamous cell carcinoma (SCC) at days 1, 3, or 14 before surgery and observed that treatment was well-tolerated with no severe adverse events and associated with tumor necrosis.[49]

Oncolytic ability of viruses can be enhanced by utilizing fusogenic viruses. Ogawa *et al*. studied the effect of dual infection with HSV-1 mutants on human oral SCC cells by infecting human oral SCC cells with γ134.5 gene-deficient HSV-1 R849 and HSV-1 HF that has multiple mutations and induces cell fusion. Results suggested that fusion-inducing virus HF enhances the oncolytic ability of γ134.5 gene-deficient HSV-1 and provides a rationale for using fusogenic viruses as enhancing agents.[50]

Combination therapy

The combination therapy, that is, oncolytic virotherapy with chemotherapy, targeted chemotherapy, antisense therapy, antibody therapy, immunotherapy, gene therapy and/or radiotherapy found to be beneficial because of their synergistic effects to combat tumors. Taking heterogeneity of tumor tissue into account, combination therapy consisting of OVs along with other treatment modality will be helpful battling against the cancer. Combination therapies lack cross-resistance and have nonoverlapping toxicity. Immunomodulating and immunosuppressive properties of certain chemotherapeutic agents, help to deliver the virus to the tumor site. Molecular mechanisms have been elucidated in preclinical studies using various combination therapies with virotherapy, chemotherapy, radiation therapy, and various other therapies. Adenoviruses express viral protein which sensitizes the infected tumor cells to radiation and expresses E1 A which sensitizes tumor cells to chemotherapy. Chemotherapy and radiation enhances the expression of certain cellular DNA repair genes, which in turn enhances replication of HSV. Treatment with the chemotherapeutic agent like fluorodeoxyuridine inhibits cellular thymidylate synthase which stimulates mammalian ribonucleotide reductase activity and increased ribonucleotide

reductase activity enhances replication of HSV-1. Cisplatin enhanced the effect of HSV possibly because of differential mechanisms of action on viral versus cellular DNA.[51-58] Monoclonal antibodies and small-molecule inhibitors alter regulatory pathways, increase viral replication, and enhance the induction of apoptosis, thus complementing the virotherapy.

Intratumoral injections of OV together with intravenous administration of cisplastin and flurouracil in patients with head and neck cancer showed higher response rate when compared to the controls with chemotherapy alone (63% vs. 30%). Moreover, toxicities were similar to those seen with each treatment alone.[35] In other trials, no overlapping toxicities were noted when dl1520 adenovirus was given in combination with chemotherapy.[59,60]

Modes of administration

A range of delivery methods have been employed to treat tumors such as intratumoral administration, systemic administration that is, through intravenous administration, intraperitonial or intraarterial administration. Intratumoral administration provides load of viruses at the site of the tumor with direct physical restriction to enhance tumor selectivity. Several OVs have been used intratumorally to treat easily reachable solid tumors with a measure of success. Local delivery of viruses is well tolerated with few side effects like mild flu like symptoms and minor local reaction.[61] Clinical studies have shown that intratumoral mode of delivery did not demonstrate any activity against distant noninjected sites. This greatly limits the potential of treating metastatic lesions.[62] Moreover, after viral replication at the site of intratumoral injection, viruses disseminated systemically are destroyed by body's defense mechanism, ineffective in the treatment of distant metastasis.

Systemic administration provides a ray of hope in the treatment of both primary as well as distant metastasis. It also has the potential to attend undiagnosed metastatic advanced disease, or patients with inaccessible disease. Systemic delivery is associated with severe flu like symptoms; but, toxicity profile remains favorable compared with other conventional cancer therapies.[17,62]

Adverse effects and safety

Toxicity of oncolytic virotherapy is favorable when compared with the toxicity of conventional cytotoxic therapies. However, safety concerns remain regarding the use of replicating viruses for the therapy. The interactions of replicating virus with both host and environment are far more difficult to predict; therefore, precautions should be taken to minimize exposure of healthcare providers, family members, and other patient contacts. Theoretically nonpathogenic viruses may acquire new pathogenic characteristics or modified viruses may revert to wild-type. All these safety issues continue to influence the development of oncolytic viral therapy.[63,64]

Obstacles

The major obstacles to oncolytic virotherapy are immune reactions against the OVs, possibility of mutations on the OVs leading to the development of cancer and chances of infection of host, hypoxia, physical barriers (e.g. normal stroma) and clearance and resistance mechanisms that may develop within the tumor milieu. Moreover, the mode of administration also plays an important role in accessibility of OVs in tumor site. Intratumoral administration of OV does not spread to other tumor sites. Efficient spread of viruses throughout the tumor may be hampered by physical barriers within the tumor micro-environment. Systemically administered virus is highly susceptible to the host immune system and may impede the therapeutic potential of virotherapy. Blood cells, complement, antibodies, and antiviral cytokines, frequently inactivate circulating viruses. However, several efforts have been made to prevent, if not otherwise circumvent, the effects of viral infection and improve the efficacy, safety, and applicability of virotherapy. Antibodies which frequently inactivate circulating viruses can be addressed by formulating the virus in liposome or collagen matrices or by using immune suppressants. Viral infections typically produce inflammatory effects, resulting in the immune system activation, tissue damage, and poor viral replication, which can be addressed by immune suppressants and antiinflammatory treatments.[65] Viruses can be modified to provide tumor selectivity and minimize toxicity, but antitumor potency of OVs will be reduced.[64] In order to enhance the cytolytic properties of the therapeutic virus as well as increasing tumor selectivity "transgene" approach has been attempted. For example, recently developed adenoviral vectors include not only an E1 deletion, which allows the virus to replicate its DNA only in tumor cells, but also a genomic rearrangement that places a reporter or a suicide transgene downstream of a constitutive promoter.[66] Another significant issue is the genomic stability of OVs and possibility of development of undesirable generation of unwanted viral strains leading to the development of another pathology, especially cancer. For example, viruses that carry deletions in the HSV 34.5 gene can replicate preferentially in cells that have elevated RAS activity.[67] In culture, however, suppressor mutants emerge that suppress the effects of this mutation, thus warning researchers that DNA rearrangements, mutations and recombination can occur with any OVs *in vitro* and *in vivo*.[68]

Future directions

Future of virotherapy is bright. Research is now concentrating on means of overcoming obstacles that are normally encountered during virotherapy. Failure to achieve effective viral delivery to targeted tumor beds and the virus-neutralizing mechanisms of the host immune system deter the therapeutic potential of virotherapy. One upcoming approach to deal with these shortcomings is to use the stem cell-based carriers to deliver the virus and shield it from immunosurveillance. Stem cells exhibit tropism for neoplasms and have unique migratory qualities enhancing

viral delivery and ultimately maximizing the therapeutic potential of oncolytic virotherapy. Stem cells also act as immune suppressant, which allows therapeutic viruses to be hidden from host defense mechanism. Stem cells also suppress the local inflammation during virotherapy, thus allowing the OV to replicate and kill tumor cells.

Conclusion

Oncolytic virus as anticancer agents have much to offer. More and more OVs will be available for clinical oncologists, which can be used alone or in combination with other agents (such as chemotherapy/radiotherapy) to treat head and neck cancer. At present there is not much evidence to prove that virotherapy is good or better than conventional approach for the treatment of cancer. Nevertheless, this field continues to advance, and new approaches are brought forward for evaluation. Cancer as an illness has refused to succumb to various advancements in the therapeutic approaches. So let's hope that OVs have the potential to subdue this indefinable illness.

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