



RESEARCH ARTICLE

Oncolytic Viruses and Their Application to Cancer Treatment

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Abstract

Great progress has been achieved in the development of oncolytic viruses (OVs). Oncolytic therapy has become a feasible and effective treatment or supplementary method to cure cancer. This review summarizes the general condition of oncolytic therapy.

Keywords

Oncolytic virus, Oncolytic therapy, Cancer treatment, Clinical trial

Introduction

Early in the 20th Century, a case was reported in The Lancet, that a notable decline in abnormal leucocyte of a female chronic-leukemia sufferer was seen, and resulted in her unexpectedly improved condition, after her accidental infection of influenza virus. In 1912, Italian doctor Deface found that tumors of patients with cervical cancer would spontaneously shrink or regress if inoculated attenuated rabies vaccines. And this has raised the curtain on oncolytic virus therapy for tumors. Several more women have injected the same vaccine for treatment subsequently. However, all the patients died of the recurrence of cancer in the end.

Researchers started to experiment with mutant natural attenuated virus strains for treatment study upon cancer cells, after realizing that viruses might have inhibiting effects on tumors. West Nile viruses and adenoviruses were largely used for oncolytic therapy in the 1950s. In the 70s, reports claimed that varicella-zoster viruses' infection could ease acute lymphoblastic leukemia. Besides, measles viruses were found effective in curing leukemia, Burkitt lymphomas and Hodgkin lymphomas, etc.

But the mechanism remained unknown and therefore cannot be properly used for tumor treatment. Also, scientists were a lack of tools for modifying more effective virus strains. Whereas chemotherapy was comparatively safer and more well-known, doctors preferred chemotherapy more. Thus, oncolytic therapy was always at the secondary position in the cancer study.

The feature and mechanism of various kinds of virus genes are gradually known as the development of virology and genetic. Advancement in techniques like genetic engineering allows people to take directed operation and modification upon virus genes, so as to manipulate virus behavior and function. Directed operation and modification have been taken upon some specific viruses since the 1990s. Over ten kinds of oncolytic viruses aiming at varied types of cancers are now in different preclinical trial phases. China approved world's first oncolytic virus therapy for cancer treatment in 2006, by using H101 for cancers of the head and neck [1].

Mechanism

a) Viruses interact with specific cell-surface receptors. As tumor cells overexpressed these proteins than normal cells did, the virus will probably infect the tumor cell.

b) After binding to the cell surface receptor, the virus is internalized by endocytosis or membrane fusion, releases its genome into the cell. Replication and viral gene expression vary according to the type of the virus. It can take place entirely in the cell cytoplasm (such as for vesicular stomatitis virus), or in the nucleus and

cytoplasm (such as for adenovirus). In either case, viral gene expression and synthesis of viral proteins largely depends on cellular machinery. Viral gene expression and replication trigger cellular antiviral defenses, such as apoptosis, which are often inactivated in tumor cells. Expression of viral proteins will eventually lead to immune-mediated lysis of infected cells. Lysis is directed by CD8+ T cells, which recognize viral peptide epitopes that are presented by major histocompatibility complex (MHC) class I molecules on the surface of the infected cell. Also, lysis may be triggered because of an overwhelming amount of budding and release of progeny virions from the cell surface, or by the activation of apoptosis during the course of viral replication and gene expression [2].

Some Oncolytic Viruses That Been Tested In Preclinical Trials

Ad(adenovirus)

At present, the main strategy of modifying adenovirus's tumor targeting and transfection efficiency is to make use of some abnormal performance of expression of tumor biology. One method is partly or entirely deletion of the gene which are unnecessary in the replication of tumor cells. Another one is to modify the necessary genetic expression during virus replication with tumor-specific promoter or enhancer [3].

ONYX-015 modified by American Onyx Company is an E1B-55K-deleted adeno-virus. Onyx-015 lacks the E1B-55K gene product, which is normally required for degrading the cellular p53 protein during viral infections, allowing it to only replicate in and destroy cells that lack p53, such as tumor cells. At present, ONYX-015 has the most complete and detailed data in clinical trials which involve many solid tumor-like cancers of the head and neck, pancreatic cancer, malignant gliomas, colorectal cancer, prostate cancer, ovarian cancer. Many basis and clinical research have been done on the intent to enhance the replication specificity of AD in tumor and the activity which required in organism against tumor. For instance, the use of Ad alone or co-expression of GM-CSF, IL-12 and IL-18 and other immunomodulatory genes in order to promote the anti-tumor activity of the body. Insertion of the telomerase promoter to enhance the replication selectivity or transform adenovirus. Ongoing clinical trials now include CGTG-102(with expression of GM-CSF) treat advanced solid tumors alone or with cyclophosphamide administration intratumor or intravenous. DNX-2401 (Delta-24-RGD-4C) treat glioblastoma and neuroglioma. (NCT00805376;NCT01582516). Intravesical instillation treat bladder cancer. (NCT01438112).

Reovirus

Reovirus infection occurs often in humans, but most cases are mild or subclinical. Reoviruses can also affect the gastrointestinal system (such as Rotavirus) and

respiratory tract. The virus can be readily detected in feces, and may also be recovered from pharyngeal or nasal secretions, urine, cerebrospinal fluid, and blood. Their role in human disease or treatment is still uncertain, but demonstrate a certain oncolytic property. Though reoviruses' molecular mechanism of selective infection and tumor cell destruction is remaining to be studied, it's generally considered that the activation of RAS signal pathway plays a key role in selective oncolytic effect mediated by this virus.

MV(measles virus; paramyxovirus)

Many researches have been done to prove the killing activity of MV on prostate, mesothelial, ovarian cancer. Heinzerling's research proved it. In a phase I dose escalation trial, 5 patients with CTCL received 16 intratumorally injections of live MV, Edmonston-Zagreb vaccine strain. The well tolerated treatment with MV resulted in clinical responses. Evaluation of biopsies, by immunohistochemistry and RT-PCR demonstrated local viral activity with positive staining for MV NP protein. All patients demonstrated an increased anti-measles antibody titer after therapy. One of the treated lesions completely disappeared [4].

Mayo Clinic has developed MV (MV-CEA & MV-NIS) that carry the gene that coding carcino embryonic antigen and sodium iodide symporter. MV-NIS is engineered to express the marker peptide carcinoembryonic antigen to permit real-time monitoring of viral gene expression in tumors. Patients with Taxol and platinum-refractory recurrent ovarian cancer and normal CEA levels were eligible for MV-CEA phase I trial. Twenty-one patients were treated with MV-CEA i.e.14 of 21 patients responded dose-dependent stable disease. Five patients had significant decreases in CA-125 level [5]. There are 5 Phase 1 clinical trials carrying on by Mayo Clinic now (NCT00408590, NCT00390299, NCT01503177, NCT01846091, NCT00450814), involving ovarian cancer, peritoneal cancer, glioblastoma multiforme tumors, malignant pleural mesothelioma, head and neck squamous cell carcinoma and multiple myeloma. Routes of administration include intratumorally, intrapleural, intraperitoneal or residual tumor resection cavity injection. Therapy options are alone or in combination with cyclophosphamide treatment [5].

HSV-1 (Herpes Simplex Virus 1)

HSV-1 is attractive for cancer therapy because of the following characteristics: (a) It infects a broad range of cell types and species, killing tumor cells at a relatively low multiplicity of infection (MOI); (b) The infection can't be affected by the antibody in blood which makes repeated injection won't weaken its efficiency; (c) The well-characterized large genome (152 kb) contains many nonessential genes that can be replaced (up to 30 kb) with multiple therapeutic transgenes; (d) Many antiherpetic drugs are available as a safeguard against

unfavorable replication of the virus; and (e) The virus remains as an episome within the infected cell, even during latency, precluding insertional mutagenesis [6].

G207 was constructed as a *second-generation* vector with both copies of $\gamma 34.5$ deleted and the *ICP6* gene inactivated. *ICP6* encode a large subunit of ribonucleotide reductase. The enzyme is the key and rate limiting enzyme of DNA synthesis and repair, which plays a crucial part in DNA replication and amplification. Therefore, the second generation obtained tumor cell targeting with double insurance [7].

G47 Δ is a *third-generation* vector that was constructed from G207 by deletion of the *ICP47* gene, which normally blocks MHC class I-mediated antigen presentation in infected cells. Consequently, human melanoma cells infected with G47 Δ expressed higher levels of MHC class I on their surface, compared to G207-infected cells, resulting in enhanced stimulation of tumor-infiltrating lymphocytes [7]. G47 Δ is safer and more effective, it has better performance in treating malignant brain tumors, prostate cancer and breast cancer. A considerable result in suppression and oncolytic effect in metastatic breast cancer, nasopharyngeal cancer, liver cancer and thyroid cancer treatment [8].

NDV (Newcastle-disease Virus)

In 2009 Schumacher [9] observed a strong inverse correlation between the susceptibility to infection and the basal expression of the antiviral genes RIG-I, IRF3, IRF7 and IFN- β . A strong expression of these genes can explain the resistance of normal cells to NDV infection and a weak antiviral gene expression the broad susceptibility of tumor cells. Thus, NDV can massive amplify in the tumor cell and specifically kill tumor cells without infecting normal cells. What's more, many of tumor cells have a higher expression of sialic acid, which is a receptor of NDV. Though the combination with the widely distributed sialic acid residues on the cell surface, NDV can get into cells and thus kill various kinds of tumor cells. NDV mediates apoptosis mainly through the endogenous apoptotic pathway and does not rely on the

involvement of the apoptotic pathway of interferon. In 2010 Bier [10] performed a siRNA-based screen of genes, which are known or predicted to participate in membrane trafficking/remodeling, to reveal Ras-related or Ras-independent NDV-sensitizers in the tumorigenic RT3 K1 cells that may also be drivers of tumorigenesis. Rac1 is an essential protein for efficient replication of oncolytic NDV in the tumorigenic cells.

With the reverse genetics' technology matures, the technology began to be applied to optimize the effect of oncolytic Newcastle disease virus. By reverse genetics technique recombinant NDV, can express an exogenous tumor-killing factor, with excellent tumor-killing ability, and achieved a good therapeutic effect in clinical trials.

VV (vaccinia virus; poxvirus)

Vaccinia virus played a decisive role in helping the human smallpox virus. With the rapid development of molecular biology, virology, immunology and cancer genetics, vaccinia virus becomes a great choice due to its effective infection to a wide range of cells, highly immunogenic, the ability to accommodate a large amount of gene inserted and correctly express them and cytoplasmic replication without the possibility of chromatin integration. Meanwhile, as the longest and most widely used virus in human history, a complete study on it provided a convenient further use. The main uses of vaccinia virus are (a) As a delivery vector for cancer; (b) As a carrier of vaccine for immune regulatory molecules and tumor associated antigens in cancer immunotherapy; (c) As oncolytic media replicate in selected cells and lead to cell lysis. The main method to treat cancer with vaccinia virus is making tumor cells originally disguised with specific surface antigens re-assigned to the targeted clusters through the partial activation of the stronger immune response, and then eliminated by macrophages [11]. Table 1 summarized the above oncolytic virus details and their development status (Table 1).

Advantages and Disadvantages between Oncolytic Therapy & Traditional Therapy

Table 1: Clinical development of oncolytic viral vectors.

Virus	Description	Mechanism	Clinical Condition
Adenovirus	medium-sized (90-100 nm), nonenveloped (without an outer lipid bilayer) viruses with an icosahedral nucleocapsid containing a double stranded DNA genome	tumor antigens targeting, conditionally replication	ColoAd1 from PsiOxus Therapeutics has entered Phase I/II clinical study with its oncolytic vaccine. ONYX-015 are used in combination with the standard chemotherapeutic agents cisplatin and 5-fluorouracil to combat head and neck tumors. the Chinese State Food and Drug Administration approved H101, an oncolytic adenovirus similar to Onyx-015 (E1B-55K/E3B-deleted), for use in combination with chemotherapy for the treatment of late-stage refractory nasopharyngeal cancer.

Reovirus	non-enveloped viruses' range between 70-80 nm, dsRNA composed of ten segments, 87 species in this family, divided among 30 genera. Most infections are mild or subclinical.	selectively infects RAS-transformed cells	Reovirus phase I clinical trials have shown indications of efficacy, and several phase II/III trials are ongoing at present. Reovirus's extensive preclinical efficacy, replication competency, and low toxicity profile in humans have placed it as an attractive anticancer therapeutic
Measles Virus	single-stranded, negative-sense, enveloped (non-segmented) RNA virus of the genus <i>Morbillivirus</i> within the family <i>Paramyxoviridae</i> .	tumor antigens re-targeting, higher CD46 densities typical of tumor cells, infection leads to extensive cell fusion.	Phase I conducted Two patients with glioblastoma multiforme have so far been enrolled into the intracerebral MV-CEA study and six patients with multiple myeloma have been enrolled for intravenous administration of MV-NIS at two dose levels
Herpes Simplex Virus 1	large double-stranded, linear DNA genome enveloped viruses	Only replicates in tumor cells	Phase I clinical trials of three vectors, G207, 1716, and NV1020, are either ongoing or completed, with no adverse events attributed to the virus. These and other HSV-1 vectors are effective against a myriad of solid tumors in mice Interim overall survival data from Amgen's phase 3 trial of a genetically-attenuated herpes virus suggests efficacy against melanoma.
Newcastle-disease Virus	contagious bird disease a negative-sense, single-stranded RNA virus	selectively replicates in interferon-defective cells	In 1999, promising results were reported using an attenuated strain of the Newcastle virus In 2006, NDV-HUJ, which showed promising results in 14 glioblastoma multiforme patients. In 2013, NDV may prove to be an attractive oncolytic agent for cancers resistant to multiple chemotherapeutic agents.
Vaccinia Virus	large, complex, enveloped virus belonging to the poxvirus family. Linear, double-stranded DNA genome approximately 190 kbp in length, and which encodes approximately 250 genes. Roughly 360 × 270 × 250 nm, approximately 5-10 fg.	Gain access to tumor through vascular leakiness	phase I–II clinical trials have been performed with IFN-β in patients with brain tumors and other metastatic solid tumors. overall efficacy was limited and transient, and significant systemic toxicities limited further dose escalation combining IFN-dependent cancer selectivity with IFN-β expression to achieve tumor-specific replication, IFN-β gene expression, and efficacy following systemic delivery in preclinical models.

OVs have many features that make them advantageous and distinct from current therapeutic modalities: (i) OVs often target multiple oncogenic pathways and apply multiple means for cytotoxicity which means the generation of resistance is at a low probability (not seen so far); (ii) OVs replicate in a tumor-selective manner and are non-pathogenic, in fact, only minimal systemic toxicity has been detected; (iii) Virus dose in the tumor increases with time due to virus amplification, which is opposed to classical drug pharmacokinetics that decreases with time; (iv) Safety features can be built in, such as drug and immune sensitivity. These features could result in a very high therapeutic index [12].

Meanwhile, viruses can trigger several spectator mechanisms to kill uninfected cancer cells. OVs can infect tumor pericytes, which elicits a constrictive inflammatory response that slows blood flow to the tumor, or OVs help present tumor associated antigens to the immune system and engage antitumor immunity [13].

However, the crucial problem over oncolytic therapy is the efficiency of the drugs delivery to the specified location. There are two main ways of administration. Intratumorally injection requires higher techniques and it's useless due to tumor's systemic metastasis and diffusion growth. Systemic intravenous injection is easier to implement and aims at several tumor, but it also has many disadvantages. (a) The existence of non-immune serum which is pre-existing or accumulated through multiple administrations can kill the viruses or cause sequestration in the liver. (b) Lack of effective extravasation pathway to transfer from the internal environment into the cell. (c) Cell carriers (i.e., mesenchymal stromal cells, myeloid-derived suppressor cells (MDSCs), neural stem cells, T cells, cytokine-induced killer cells, or irradiated tumor cells) can shield virus from neutralization and facilitate virus delivery to the tumor.

Therefore, the effectiveness depends on the cell

phenotype, permissiveness to virus infection, tumor homing ability, and transfer of infectious virus to tumor cells [12].

Development Prospect

As the situation stated above, there are several recent approaches to improve oncolytic virus delivery.

Targeting the Tumor Cell Surface

During tumor evolution, various genetic and epigenetic events result in the unique display or overexpression of so-called 'tumor antigens' on the surface of malignant cancer cells. As cell surface recognition and virus entry are the first procedure in a productive viral infection, engineering a virus which can specifically recognize the tumor cell surface would restrict replication of a potent oncolytic virus to malignant cancer cells [2].

Exploiting the Tumor Microenvironment

Given that virally encoded receptors are highly evolved proteins, an alternative approach is to use the *in vivo* tumor environment to augment selectivity without the complete re-engineering of an already efficient system. For example, subtle alterations in the fusion (F)-protein of measles virus allow it to be processed to an active form only in the protease-rich tumor microenvironment. The F-protein of measles virus facilitates viral entry into cells by mediating fusion of the viral and cellular membranes [2].

Non-enveloped viruses is another option. For example, reovirus normally infects cells of the gastrointestinal tract, where proteases can convert the non-infectious reovirus into an infectious form called the intermediate sub-viral particle (ISVP). When given intravenously, reovirus is not efficiently processed to the infectious form. However, it is possible to select variants that have been converted into ISVP by the action of proteases that are overexpressed in the tumor microenvironment. These selected reoviruses have been shown, *in vivo*, to selectively infect and kill malignant lymphoid cells that produce a protease-rich microenvironment [14].

'Naturally Smart' Viruses

Viruses have evolved to gain access to the cell through binding to proteins that are displayed on the plasma membrane and that often have crucial roles in regulating normal cell proliferation, homeostasis or adhesion [2]. Recognizing this, Darren Shafren's group have screened a collection of picornaviruses in a search for viruses that preferentially infect tumor cells, based on their overexpression of natural virus receptors [15].

Tumor Growth and Innate Immunity

Innate immunity is a non-specific defense mechanism which is triggered immediately after pathogen detection and does not develop immunological memory

for antigens. OV's can be modified to make better use of innate and adaptive responses to eliminate tumor cells [2].

One strategy to develop oncolytic therapeutics is to select or design viruses that are especially sensitive to the antiviral properties of interferons. Such viruses should have their replication strongly suppressed in interferon-responsive normal tissues but still be able to amplify in interferon-nonresponsive tumor cells. The tumor-selective oncolytic activity could be achieved by deleting or attenuating gene which encode these anti-interferon-gene products [16].

Another common defect in tumor cells that might make them susceptible to oncolytic virus activity involves the downregulation of p53 or its downstream targets. Mice with supernumerary copies of the normal Trp53 gene are both more resistant to VSV infection and have a decreased incidence of tumor formation [17].

Another design strategy for oncolytic viruses would be to delete viral anti-apoptotic genes, creating mutants that only replicate in apoptosis-deficient tumor cells [18].

Diplomatic Immunity

The mammalian adaptive immune system has evolved to restrict the replication and spread of invading pathogens. For oncolytic virus-based therapeutics, this is a double-edged sword. On the one hand, these defense mechanisms block the delivery and/or spread of oncolytic viruses. On the other hand, viral stimulation of the adaptive immune system seems to activate anti-tumor immune surveillance systems, increasing the effectiveness of oncolytic virus therapy. Oncolytic viruses not only mediate direct tumor oncolysis, but also, in combination with their inherent adjuvant properties, induce or reactivate cancer immune surveillance programs. These phenomena indicate that: First, it might not be crucial for the oncolytic virus alone to completely eradicate a tumor to be therapeutically effective. Rather, if the virus can quickly establish a tumor-specific infection, this will lead to a localized inflammation, *in situ* cytokine production and ultimately an anti-tumor immune response. Second, oncolytic viruses that have been engineered to produce immune stimulatory factors on infection of tumor cells may be more effective therapeutics [2].

Conclusion

Study on oncolytic viruses not only contributes to cancer treatment, but also reveals much upon how cells regulate genetic expression [2]. In spite of considerable improvement in the last two decades, further progress is needed in oncolytic virus therapy for cancers. Methods include equipping oncolytic virus with therapeutic genes; optimizing traditional chemotherapy and radio-

therapy with combination of oncolytic therapy; adjust body immune response to achieve minimal antiviral effect with maximum anti-tumor effect.

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