An overview of cancer virotherapeutics

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History of the use of virotherapeutics in cancer

Cancer, a group of more than 200 diseases characterized by uncontrolled cell division, is a worldwide health issue responsible for the death of millions of people each year. In America, it has been estimated that cancer accounts for one in four human deaths; nearly 570,000 deaths of Americans in 2010 alone.[1] Although the existence of cancer has been known for centuries, most types of cancer still have a poor prognosis and result in mortality. However, the five-year cancer survival rate has increased to 68% as of 2005 from only 50% in 1997.[1] Currently, the main treatments for cancer involve chemotherapy, radiation therapy, surgery, or transplants.[2,3] However, mortality rates of this disease are still extremely high and many therapeutics are ineffective. Therefore, these various issues have led to research into the development of atypical therapeutics, such as virotherapies, which utilize genetically modified viruses to seek out and destroy diseased cells without harming healthy cells. Virotherapies are mainly developed to selectively kill cancerous cells, but can also be used for non-cancerous tumor lysis. Viral vectors used for delivery of genetic material are additionally used to treat a wide range of other diseases, such as immunodeficiencies.

Although use of virotherapeutics is still a fairly recent method of cancer treatment, the idea of using virotherapies as a way to eradicate tumors has been in existence for more than a century. Throughout the late nineteenth and early twentieth centuries, scientists worldwide reported remissions of cancer caused by infections with viruses or even by vaccinations.[3-5] In 1912, a physician reported an 8-year regression of cervical carcinoma in a woman who had been given a live attenuated rabies vaccine after being bitten by a rabid dog.[5]

Beginning in the 1920s, a variety of viruses have been tested for their oncolytic properties.[6,7] In the early 1950s, vaccinia virus became the first virus proven to have definite oncolytic activity against tumors.[6] By the 1960s, several other viruses had proved to be exceptionally promising as cancer reducing agents.[8] However, this treatment was not without its flaws. The effects of early cancer virotherapeutics showed a lack of clinical efficacy by its mostly unimpressive results. Additionally, serious toxicity of these therapies could be seen in some patients. For these reasons - as well as the enthusiasm for the new development of chemotherapy - the majority of research into cancer virotherapeutics lagged until the 1990s.[9]

The end of the 20th century brought with it the advent of genetic engineering and molecular virology: two areas of research that allowed for the resurgence of interest in the field. Research discoveries allowed for the modification of the viruses themselves in order to enhance their cancer selectivity and anti-tumor potency while minimizing toxicity.[10] In 1991, the first genetically engineered virus, a thymidine kinase negative mutant of herpes simplex virus-1, *dl*sptk, was reported to successfully inhibit cancerous tumor growth and to prolong host survival.[11] By 1996, an adenovirus mutant, ONYX-015, was shown to be more tumor-specific than was the wild-type adenovirus.[12] Soon after, it became the first genetically engineered cancer virotherapeutic to be enrolled in clinical trials.[13] Six years later, nearly 10 cancer virotherapeutics had neared or been entered in clinical trials. In 2005, China approved Adenovirus type 5 mutant H101, a mutant nearly identical to ONYX-015, for the treatment of head and neck squamous cell carcinoma.[14] China thus became the first country to approve a virotherapeutic for cancer treatment.

Development of 21st century cancer virotherapeutics

Virotherapeutics development is generally based upon both the molecular mechanisms of viral infection and cellular actions. However, manipulated viruses have now been shown to have a much higher efficacy than their wild-type counterparts. In this fashion, virotherapies can now be designed to be virocentric or immunocentric, depending on the type of cancer and the desired mechanism of the therapy. Virocentric therapeutics investigators view direct tumor cell lysis as the most important aspect of efficacy when treating cancer, whereas immunocentric therapeutics view activation of the immune system response to the cancer cells as most important. These two mechanisms allow for improved design of virotherapies, generally based on the immunogenicity and peculiarities of the cancers being treated.[15]

Currently there are several types of cancer virotherapeutics in development. These can be separated into five groups: direct cell lysis due to viral replication, direct cytotoxicity of viral protein, induction of anti-tumor immunity, sensitization to chemotherapy and radiation therapy, and transgene expression as listed in Table 1.[15] Adenoviruses and herpes simplex viruses are some of the most commonly manipulated potential virotherapeutic agents, with viral strains serving as vectors able to fit into all five categories. However, viruses of other families are being assessed for potential roles as cancer therapeutics. Table 2 shows some of the most common viruses currently being investigated as potential virotherapeutics.[16] This list is by no means complete as there are dozens of viruses being assessed for use as therapeutics. However, the majority of these viruses, although showing promise in preliminary studies, using animal models, have not been developed for further use.

Virotherapeutic mechanisms of tumor selectivity

Tumor selectivity is important when creating a cancer therapeutic. Mechanisms of virotherapeutics can be categorized into four main groups: inherent tumor selectivity,[18] viral gene inactivation,[19,20] transcriptional targeting,[21] and transductional targeting.[22]

Tumor selectivity

Virotherapeutics with inherent tumor selectivity are wild-type viruses that preferentially infect and replicate in cancer cells. Preferential infection by these viruses is due to physiological alterations of these cells, which cause an increased rate of division of the cells and an evasion of the host immune response. Although for use as virotherapeutics most viruses are genetically engineered to be tumor selective, various viruses, such as the Sindbis virus, Newcastle disease virus, and measles virus have been used as virotherapeutics without any genetic alterations. Most genetically non-engineered virotherapeutics are either paramyxoviruses or togaviruses.[23]

An example of a virotherapeutic with inherent tumor selectivity is the Type 3 reovirus; a non-enveloped double stranded RNA virus belonging to the *Reoviridae* family. Reoviruses infect humans (and other vertebrate hosts and even some invertebrate hosts), but infections tend to be asymptomatic or restricted to mild respiratory and gastrointestinal illnesses.[24] In the late 1970s, *in vitro* testing of the Dearing strain from Type 3 reovirus showed that it preferentially killed cells transformed by simian virus 40.[25] Later research showed reovirus oncolysis to be associated with Ras signaling pathway activation in transformed cells. RAS (a family of proteins originally found in rat sarcoma cells) promotes cell proliferation, transformation, and metastasis.[26,27] Additional studies showed that activation of RAS could potentially enhance reovirus action by increasing viral uncoating, apoptosis-dependent release, and infectivity.[28] Clinical trials have shown that an altered form of Dearing strain Type 3 reovirus known as REOLYSIN is a functional antitumor agent that is well tolerated and not overly harmful to humans.[29]

Viral gene inactivation

Viruses that have had certain genes inactivated are one of the most common types of virotherapeutics. Viruses such as these have genes that promote cell growth and evasion of antiviral responses. However, these genes are unnecessary for infection and growth in cancer cells since these cells already have mechanisms of increased proliferation and tend to be defective in generating antiviral responses.[23] In this fashion, viral genes are unnecessary for virotherapeutic use, and can consequently be used to improve tumor selectivity. Several viruses, such as the vaccinia virus, herpes simplex viruses, and adenovirus have been used as gene inactivated virotherapeutics.[23] All of these viruses have gone to clinical trials. A strain of herpes simplex virus with a gene for GM-CSF, a cytokine, known as Oncovex (OncoVEXGM-CSF; Amgen Inc.) is currently completing phase III trials for melanomas and squamous cell carcinomas of the head and neck.[30]

An example of a virotherapeutic with gene inactivation is the human group C adenovirus, a double stranded DNA virus of the family *Adenoviridae*. Adenoviruses rarely cause serious diseases in humans and are most typically known as a cause of the common cold.[30] More than 50% of the human population has been infected with or exposed to adenovirus serotype 5: one of the two main serotypes of adenovirus used as vectors in virotherapeutics.[31] Cancer-specific adenoviruses have been generated in several ways. The first adenovirus deletion mutant used as a virotherapeutic, ONYX-015, lacked the gene encoding E1B-55kD, which binds to and inactivates a tumor suppressor gene known as p53.[12,23] This deletion was made in order to promote selective replication of p53 defective tumor cells. Adenovirus deletion mutants can also have multiple deletions, such as mutant CB1, which has a deletion of E1B-55kD, as well as of CR-2, a gene that encodes complement receptors.[33] Although certain adenoviruses have been shown to be extremely safe, other adenoviruses have caused serious issues, such as toxic shock.[4,6]

Transcriptional targeting

Virotherapeutics are sometimes transcriptionally targeted in order to enhance cancer cell specific viral replication.[23] This method involves specific promoters to control viral genes necessary for viral replication. Over the last few decades, more than 30 promoters have been discovered as methods for transcriptional targeting in virotherapies. Tissue and tumor specific promoters can be divided into two main types: tissue/tumor type-specific promoters, which are active in specific types of tissues or tumors, and pan-cancer specific promoters, which are active in various tumor types but are inactive in normal cell types.[10,34] Transcriptional targeting has most commonly been applied to adenoviruses, parvoviruses, and herpes simplex virus type 1.[35,36] In each case, different combinations of viral genes have been targeted, and transcriptional targeting is often combined with other methods to enhance tumor selectivity.[37]

Transductional targeting

Certain virotherapeutics are altered prior to virus entry through transductional targeting. By this method, virus entry can be modified to only recognize cancer cells so that replication is restricted to them. Transductional targeting can be accomplished in various ways, such as through pseudotyping, use of adaptors, and genetic incorporation of targeting ligands. Transductional targeting is most often used to increase cancer cell specificity in herpes simplex virus type 1, measles virus, vaccinia virus, and adenoviruses.[10,38]

Tumor specificity and virotherapeutic potency

All cancer virotherapeutics strive for increased tumor specificity, which is mediated through one of the four main mechanisms of tumor selectivity. Once obtained, anti-tumor potency is analyzed to determine the clinical efficacy of the therapeutic. In this, virotherapeutics mediate tumor destruction through intrinsic anti-tumor activity, immune responses, expression of therapeutic genes, and sensitization to chemotherapy or radiation therapy.[10] Intrinsic anti-tumor activity replicates and destroys cancer cells through apoptosis or necrosis. Virotherapy-triggered immune responses involve induction of cytokines, release of tumor-associated antigens, or activation of tumor-infiltrating dendritic cells within tumors.[39] Expression of therapeutic genes occurs through use of virotherapeutics enabled with genes that allow for increased tumor specificity and viral replication.[40] Sensitization of conventional cancer therapies, such as chemotherapy or radiotherapy, is often necessary since - due to their lack of sensitivity - these therapies often become ineffective in treating advanced stage patients.[40] However, virotherapeutics can enhance the response to these therapies, as has been shown with viruses such as measles virus. Additionally, virotherapeutics tend to be enhanced when combined with typical therapeutics such as the above mentioned and show high increases in efficacy.[41]

Difficulties and challenges of cancer virotherapies

Like most cancer therapeutics, virotherapeutics have drawbacks. In respect to virotherapies, vector-related issues can be narrowed into three main categories: (1) low infectivity, (2) vector agglutination to antibody, and (3) negative immune responses; two further subdivisions of this can be made as cytotoxic T lymphocyte (CTL) toxicity from the vector and cytokine production resulting in viral inhibition. CTL toxicity can eliminate cell populations in ways that are detrimental to virotherapy treatment, preventing the full effect of viral replication on the host. Additionally, production of cytokines, immunomodulating proteins, resulting in viral inhibition is the result of viral infections that cause inflammatory effects. Both CTL toxicity and cytokine production resulting in viral inhibition can be prevented by use of immunosuppresants and specific vector design in order to minimize immune responses that are detrimental to treatment. Cytokine production can also be prevented through anti-inflammatory treatments.[42,43]

Low infectivity is often caused by poor viral transduction. Vector agglutination is caused by antibody inactivation of circu-

lating viruses. Although entirely different issues, low infectivity and vector agglutination to antibody have similar corrective methods. Both can be addressed through use of specific vector design or use of liposomes, which are vesicles used for administration of nutrients as well as therapeutics. Vector agglutination by antibody can also be corrected through use of collagen matrices and immune suppressants, while low infectivity can be fixed through protein coat alterations and bidirectional antibodies.[42]

Lastly, for virotherapies to be successful, viral infection of cancer cells must exceed growth rates of uninfected cancer cells. Therefore, the efficacy of each virotherapy must be assessed as they may need to be incorporated with a preliminary treatment, such as chemotherapy or surgery.[43] Efficient delivery of the vector also plays a major role in the functionality of virotherapy; systemic injections require 1000x the viral load needed to obtain a desired result in comparison to intra-tumor injections.[44] All these issues must be addressed in order to create a fully functional virotherapeutic.

Safety issues regarding use of cancer virotherapies

Although cancer virotherapeutics have great promise, the field is hardly risk-free. During the first wave of interest in virotherapeutics that led to clinical trials in the late 1940s and early 1950s, adverse effects were common. These included, encephalitis, fever, bleeding, and other more mild signs. One death, in the case of a 1949 clinical trial for Hodgkin's disease, even resulted in a death after injection of the hepatitis B virus.[9]

Since the second wave of interest in virotherapeutics however, data accumulated has shown that virotherapies are mostly safe. The most common adverse effects usually being flu-like symptoms and fever.[3,10] However, several clinical virotherapy trials have resulted in serious adverse effects and death. For example, in May 2002, a 55-year-old male with renal carcinoma metastatic to the lungs died of respiratory failure five days after an intravenous dose of PV701, a replication competent strain of Newcastle disease virus: His death was possibly due to rapid tumor lysis.[45] Additionally, clinical trials which use virus vectors tend to influence the fate of virotherapeutics. In September 1999, a teenager died of toxic shock after receiving an adenovirus vector to treat ornithine transcarbamylase deficiency.[46] In October and December 2002, two young boys who enrolled in a program for the treatment of X-linked severe combined immunodeficiency using a retroviral vector developed a form of leukemia, which resulted in one death. A third child in that program developed leukemia in January 2005.[46] These adverse effects highlight the most serious obstacles of tumor virotherapeutics – immune reactions against vectors and transgenes, and inappropriate insertions of vectors and transgenes that can potentially lead to further cancer-causing mutations.[43,46]

Conclusion

The field of virotherapeutics is being developed into a fairly new area of treatment, one which holds great promise. Viruses of dozens of families have potential and many are innately capable of acting as viral therapeutic agents. Regulation of tumor selectivity and consequent anti-tumor potency have been shown to be of key importance, and have proven that virotherapeutics can be used to target and destroy cancer cells effectively while leaving normal cells unharmed. Since genetic engineering and biotechnology were demonstrated to be applicable to virotherapeutics, viruses have also been manipulated in order to increase their

Mechanism	Mode of Action
Direct cell lysis due to viral replication	The virus destroys cancer cells by replicating until inhibited by the immune response or by a lack of susceptible cells.
Direct cytotoxicity of viral protein	The virus destroys cancer cells by synthesizing proteins during replication that are cytotoxic.
Induction of anti-tumor immunity	The virus takes advantage of the weak immunogenicity of cancer cells and initiates anti-tumor immune responses.
Sensitization to chemotherapy and radiation therapy	The virus functions to prevent or stop chemoresistance or resistance to other similar therapies. The virus also functions in one of the ways listed above and tends to be minimally effective on its own, but is highly effective when combined with other therapeutics.
Transgene expression	The virus is genomically altered in order to improve its efficacy and specificity in cancer cell destruction.

Table 1: Mechanisms of Anti-Tumor Efficacy in Virotherapeutics
**Adapted from reference 16.

Family	Genus	Strain/Vector Used
Adenoviridae	*Mastadenovirus*	Conditionally replicating vectors based on canine adenovirus.
Herpesviridae	*Simplexvirus*	Replication-competent vectors based different types of strains of HSV-1 and HSV- 2.
Polyomaviridae	*Orthopoxvirus*	Replication-competent vectors based on vaccinia strains WR and Wyeth.
Reoviridae	*Orthoreovirus*	Live reovirus type 3 strain Dearing (T3D).
Orthomyxoviridae	*Influenzavirus A*	Replication-competent NS1 deleted influenza A.
Picornaviridae	*Enterovirus*	Live echovirus type 1 and coxsackievirus A21. Replication-competent vectors of poliovirus type 1. Live attenuated poliovirus and bovine enterovirus.
Togaviridae	*Alphavirus*	Live attenuated Sindbis virus and replicons.
Coronaviridae	*Coronavirus*	Replication-competent vectors based on feline coronavirus and murine hepatitis.

Table 2: Viruses Used in Cancer Virotherapies**
** Adapted from Reference 17.

efficacy in treating tumors.

Many virotherapies are currently in clinical trials, which have, for the most part, shown to be well-tolerated by humans. Therapies in which virotherapeutics are used in combination with more typical therapies- such as chemotherapy, radiotherapy, and antibody therapy - have been shown to provide much more effective results. Most of the issues that prevented the field of cancer virotherapeutics from expanding now are actively being overcome. Advances in research must now focus on the most serious obstacles of the field—\–vector and transgene caused immunological reactions, as well as inappropriate insertions of vectors and transgenes that can lead to further cancer-causing mutations. However, with viruses such as Oncovex reaching completion of phase III trials and adenovirus H101 approved for cancer treatment in China, virotherapeutics will continue to be in trial and, perhaps, become available as cancer therapies worldwide.

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