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Abstract

Cancer is the leading cause of disease-related death in companion animals such as dogs and cats. Oncolytic viruses refer to those viruses that will selectively eliminate and lysis cancerous tissues without causing harm to normal tissues. Oncolytic viruses can kill infected cancer cells in many different ways, ranging from direct virus-mediated cytotoxicity through a variety of cytotoxic immune effector mechanisms. Treatment of pet cancer with advanced disease stages is very poor prognosis. Therefore, developing unique cancer therapies is essential for work synergistically in combination with the conventional treatment options. Several oncolytic viruses including canine distemper virus, adenovirus strains, and vaccinia virus strains have been used for canine cancer therapy in preclinical studies. This review is focused on the probable use of oncolytic viral agents for canine cancer therapy in the future.

Keywords: Cancer, Canine, Oncolytic virus; Oncolyis; Companion animals

Abbreviations: OVs: Oncolytic Viruses; CDV: Canine Distemper Virus; RGD: Arginine-Glycine-Aspartic Acid; CAR: Coxackie-and Adenovirus Receptor; Ad: Adenoviruses; CAV-2: Canine Adenovirus 2; MV: Measles Virus; MCT: Mast Cell Tumors; VACV: Vaccinia Virus; VEGF: Vascular Endothelial Growth Factor

Introduction

Cancer is one of the most common causes of natural death in dogs in both developed and developing countries. It is among the top deadly diseases in dogs and cats [1-3]. The incidence of canine cancer ranges from 1% to 2% and currently accounts half of the death in the canine population older than 10 years [1,4,5]. In contrast to the progress of oncolytic virotherapy in human being, there are very limited clinical trials using oncolytic viruses (OVs) for canine or feline cancer patients [6,7]. The most common forms of cancer in dogs and cats are skin, lymphoma, mammary, bone, connective tissue, and oral cancers [8,9]. Treatment of pet cancer with advanced disease stages is very poor prognosis by major traditional options for canine cancers treatment includes surgery, chemotherapy, radiation therapy, and hyperthermia. Therefore, developing unique cancer therapies is essential for work synergistically in combination with the conventional treatment options. Although many forms of canine or feline neoplasms forms are similar to their human complements in histological appearance, biologic behavior, tumor genetics, risk factors, pathologic expression, and response to therapy [10,11], it will expect that the clinical procedures used in humans will transfer to the treatment of cancer patients in companion animals. In human, several OVs including adenovirus, vaccinia virus, herpes simplex virus, Seneca Valley virus and reovirus are currently entering Phase III human clinical trials [12,13]. In contradiction of the progress oncolytic virotherapy in human, there are a small number of clinical trials using OVs for canine cancer patients [14,15]. A mouse was considered the first animal model to demonstrate full regression through viral oncolysis, Alice Moore was the first scientist tested Russian Far East encephalitis virus in some cases of mouse sarcoma 180 [16]. However, the use of oncolytic virotherapy in veterinary medicine is far from reality.
and present are also the challenges and the major obstacles to the optimal practice of oncolytic virotherapy in canine cancer patients. This review describes the most common classes of oncolytic viruses for canine cancer therapy and focuses on ways of their progress in preclinical studies with canine cancers.

**Cancer gene therapy**

Gene therapy is a field of research aiming to treat diseases caused by defective genes by altering the genomes of cells and tissues [17]. Disease entities for which gene therapy is being developed include for example cancer, cardiovascular disease, neurological diseases, hematological diseases, monogenic inherited diseases and infectious diseases. By June 2014, a total of 2076 clinical gene therapy trials had been initiated. The vast majority of these (64%) were aimed at cancer gene therapy.

**Cancer gene therapy approaches fall into four main strategies**

Insertion of a normal gene into cancer cells to replace a mutated gene: For example, a mutation in a p53 protein, which interferes with the ability of tumor cells to destruct themselves by apoptosis, is found in most of the cancers [18].

Silence a mutated gene which is activated or overexpressed in cancer cells: Such oncogenes can for example drive tumor growth, blood vessel formation, induce metastasis to other tissues, and allow for resistance to chemotherapy. Silencing can be accomplished by using e.g. small interfering RNA (siRNA) silencing technology, which has been used to specifically target, for example, tumor suppressor p53 molecules containing a single point mutation, leaving the wild-type suppressor intact [19].

Introducing genes that make cancer cells more sensitive to standard chemotherapy or for radiation treatments: Drug convertases (“suicide genes”) which can turn an inactive pro-drug into an active drug which can be introduced to tumor cells to cause cell-specific toxicity. For example, the herpes virus thymidine kinase can phosphorylate and convert non-toxic drug ganciclovir into toxic metabolites [20].

Direct cell killing with targeted viruses: After genetic engineering, oncolytic viruses selectively replicate in cancer cells leading to tumor cell destruction and oncolysis [21].

**OVs as a gene therapy for cancer**

Different approaches utilizing viruses have been used for cancer treatment for several decades. Non-replicating or replicating viruses can be used as a gene transfer vector to introduce a therapeutic gene, co-stimulatory molecule or cytokine into cancer cells or to prime lymphocytes with tumor antigens in cancer vaccine approaches [21]. The initial reports of virus-induced oncolysis date back to 1904 (DOCK) and 1912 (DE PACE), respectively, and referred tumor regression of human cervical carcinomas after rabies vaccination. In the 1920s animal experiments confirmed that viruses were capable of infecting and lysing experimental murine tumors and several studies followed in the 1950s demonstrating potent oncolysis of murine tumors by Newcastle disease virus and Influenza virus [22]. Studies of human cancer were initiated in the 1950s. Perhaps the most recognized of these investigations was one report from the National Cancer Institute in 1956, in which wild-type adenoviruses of different serotypes were injected in patients with cervical carcinoma [23]. There are two important aspects to oncolytic virotherapy; there is a direct treatment of tumors with replicating oncolytic viral vectors alone or in combination with therapeutic transgene delivery, chemotherapy, or radiation therapy. On the other hand, there is indirect increase of antitumor immunity through a modulation of the immune response, as with viral oncolysate vaccine, and tumor-protective monoclonal antibodies [24].

By June 2014, viruses were being used as vector systems in approximately two-thirds of all gene therapy trials. Out of different virus vectors, adenoviruses (23%) and retroviruses (19%) have been reported as the most (Figure 1) [21].

![Figure 1 Cancer gene therapy trials.](image-url)

Recently, a gene therapy trial that led to dramatic benefits for babies born with a fatal neuromuscular condition has raised hopes for using a similar line to treat other diseases. But a new animal study recommends that the high doses (2x10E14 genome copies/kg BW) of gene-carrying viruses used in such treatments may not always be as safe as the human clinical trial indicated. In the recent study, the disclosure of which briefly sent the stock prices of several gene therapy companies dropping yesterday, scientists injected a handful of young monkeys and pigs with many copies of adeno-associated virus 9 (AAV9), a normally harmless virus that infects neurons and is increasingly being used to ferry therapeutic genes into cells to treat neuromuscular diseases. Within days, some of the animals developed severe liver and neuron damage.

Oncolytic viruses are distinguished by their property to either inherently or after genetic modification replicates selectively in cancer cells. These viruses have multiple mechanisms to harm the host cells including direct lysis, induction of apoptosis and autophagy, expression of toxic proteins and shut-down of protein synthesis. At the end of the replication cycle, cells are destroyed and infective viral progeny is released into remaining tumor tissue. In addition to
local amplifying antitumor effect, infective viral particles are able to enter the systemic circulation and infect distant metastasis (Figure 2) [21,25]. In addition to naturally occurring oncolytic viruses such as reovirus [26]. Several human DNA and RNA viruses such as measles virus (MV), vesicular stomatitis virus (VSV), adenovirus, vaccinia virus (VV) and herpes simplex virus (HSV) have been genetically modified to selectively replicate in tumor cells, while their activity in normal cells is attenuated [25,27].

Adenoviruses

Adenoviruses (Ad) are non-enveloped, double-stranded DNA viruses of approximately 90 nm in diameter. The virus is surrounded by an icosahedral capsid consisting of penton and hexon proteins, knobbed fiber proteins extended from the twelve vertices. Each penton protein has flexible loops on its surface, featuring an arginine-glycine-aspartic acid (RGD) motif which is involved in cellular binding and internalization [28]. The virus enters the cells by binding to a high-affinity cell surface receptor with its fiber knob. Most adenovirus species have been shown to bind to coxsackie and adenovirus receptor (CAR), which triggers secondary interaction with RGD motif and cellular αvβ-integrins leading to endocytosis via clathrin-coated pits [29,30]. The life cycle of the virus can be divided into two phases separated by the onset of viral DNA replication.

Adenoviruses are one of the most commonly used vectors for cancer gene therapy. The virus was first identified in the 1950s and ever since they have been intensively studied as gene therapy vectors [31]. Adenoviridae family can be divided into 4 genera and 6 species [32], and so far 59 serotypes of human adenoviruses have been identified [33]. The various serotypes have been further classified into subgroups A-G, depending on their ability to agglutinate erythrocytes [34]. In general, adenoviruses are endemic in most parts of the world and have low pathogenicity in humans. Different serotypes have been shown to have different pathological effects but typically adenoviruses infect the epithelial cells in the respiratory and gastrointestinal tract or the eyes causing mild flu, conjunctivitis and infantile gastroenteritis [35,36].

In canine adenovirus 2 (CAV-2), by inserting osteocalcin promoter showed restricted replication in canine osteosarcoma cells. Osteocalcin promoter is active only in osteosarcoma cells and not active in other canine non-neoplastic cells. This promoter was tested as a therapeutic agent for canine osteosarcoma [37]. In addition, administration of this modified canine adenovirus to normal dogs showed only moderate virus-associated toxicity and showed therapeutic benefits in the xenograft model in killed canine osteosarcoma cells in cell culture [37,38]. The expression of heterologous genes by canine adenoviruses can lead to enhanced therapeutic activity. In canine adenoviral vector AdCD40L exhibited complete tumor regression in 5 of 19 canine melanoma patients [39].

Vaccinia viruses

Vaccinia is a complex double-stranded DNA virus, brick-shaped particles with a size of approximately 300 x 240 x 120 nm [40]. Infectious vaccinia virus particles have a lipoprotein envelope surrounding a complex core of linear double-stranded DNA (191 636 bp, encodes for ~250 genes) [41]. Vaccinia encodes all the proteins it needs for its replication in its genome, some of which have immune evading properties allowing the virus to establish infection [42]. Vaccinia virus enters the cell via fusion of viral and cellular membranes, which is mediated by entry-fusion complex [43,44]. No specific receptor to facilitate entry of the virus into the cell has yet been discovered. After the entry, viral particles are uncoated, and transcription of early genes by the viral RNA polymerase starts followed by the expression of intermediate and late genes [45].

In 1798, Edward Jenner noticed that milkmaids exposed to cowpox developed protection against smallpox [46]. Smallpox was caused by variola, a member of the poxvirus family. This outcome eventually leads to the development of a laboratory strain of poxvirus, vaccinia virus, used as a vaccine in the Smallpox Eradication Program led by the World Health Organization [47,48]. Vaccinia is a member of the Orthopoxvirus genus and is its most extensively studied member. It was the first mammalian virus to be visualized microscopically, successfully grown in tissue culture, titrated accurately, purified physically and analyzed biochemically [40]. Due to this historical role, vaccinia virus has the longest and most extensive history of use in humans of any virus and has had a major impact on the development of vaccines. Wild-type vaccinia virus has been used in hundreds of millions of humans as a vaccine for the eradication of smallpox and has shown a good safety profile as only rare serious side effects have been reported during the vaccination program [49]. Vaccinia virus has been studied as a viral vector for the development of cancer virotherapy, immunotherapies, as well as the development of next generation smallpox vaccines due to its strong safety profile and high immunogenicity [50].
Morbilliviruses

Canine distemper virus (CDV) is an enveloped, negative-sense single stranded RNA virus of the family Paramyxoviridae, closely related to human measles and rinderpest virus, that infects different cell types, including epithelial, mesenchymal, neuroendocrine and hematopoietic cells of various organs and tissues [51,52]. It is a close relative of measles virus (MV). CDV is able to infect canine lymphoid cell lines, histiocytic sarcoma cell lines, such as DH82 cells. Moreover, CDV induced an increase of apoptotic cells in neoplastic lymphocytes in vitro [53,54]. Historically, children with Hodgkin’s disease were observed to experience regression after concurrent MV infection [55]. These explanations stimulated the consideration of attenuated MV for the treatment of human lymphoma and, consequently, measles virus has revealed promising anti-tumor activity against a variety of malignant tumors in both preclinical and clinical studies [56]. Because of its similarity to MV, this finding underscore the possible relevance of CDV as an oncolytic agent and the formulation of the hypothesis that CDV represents a suitable candidate for a tumor environment induced immunogenic cytotoxicity following tumor regression in a variety of different xenograft models, even in cancer cells refractory to conventional therapy [64,65,66]. CDV-induced tumor cell lysis, even in cancer cells refractory to conventional therapy and molecular targeted therapies [62]. Furthermore, deployment of coxsackie viruses into the local tumor environment induced productive cell spread and promoted immunogenic cytotoxicity following tumor oncolysis.

Coxsackie virus

Coxsackie virus is an enterovirus belonging to the Picornaviridae family of nonenveloped viruses containing a linear, positive sense, single-stranded RNA genome. Because RNA viruses replicate in the host cytosol without a DNA phase, insertional mutagenesis is not possible. Coxsackie viruses are divided into two subgroups, A and B, based on pathogenicity in mice. At least 23 serotypes of group A and six serotypes of group B have been described. Coxsackie viruses are considered to be a minor human pathogen. Young children, aged five years and under, are more susceptible to coxsackie virus A disease, often produced by serotype A16. Infection of individuals occurs mainly via entry through exposed areas, such as the skin and mucosal surfaces (i.e., hands, feet, mouth, throat, and eyes). However, in most cases, infection is asymptomatic or elicits only mild disease associated with “common cold-like” symptoms [59-61]. Various nonengineered strains of coxsackie virus from both groups are currently being tested as single oncolytic therapeutics or in combination with conventional chemotherapy drugs.

In preclinical models, coxsackie virus B3 (CVB3) exhibited potent tumor cell lysis in a number of non-small cell lung cancer cell lines, even in cancer cells refractory to conventional radiotherapy and molecular targeted therapies [62]. Furthermore, deployment of coxsackie viruses into the local tumor environment induced productive cell spread and promoted immunogenic cytotoxicity following tumor oncolysis.

Reovirus

Reovirus, is a segmented dsRNA consisting of 10 to 12 segments, each generally encoding one protein. The virus has been broadly tested in oncolytic virotherapy of human cancers over the past decade. While its expansion in phase II and III clinical trials for treatment of human cancer patients, it has been hardly studied as an oncolytic agent in pet animals. Recently, in vitro study demonstrated for the first time that canine mast cell tumors (MCT) were highly susceptible to reovirus infection [63]. Furthermore, a single intratumoral injection of reovirus significantly regressed canine mast cell tumor xenografts [63]. However, reovirus also infected normal canine mast cells raising safety concerns.

Possible mechanisms of oncolytic virus-mediated tumor ablation

Oncolytic viruses infect the tumor cells and mediate the its regression either by direct viral lysis of tumor cells [64,65], by the destruction of the tumor vasculature [65], by induction of host antitumor immune responses [66,67] or most likely, a combination of these mechanisms [68,74] (Figures 3 and 4). The increase of immune cells infiltration (neutrophils, macrophages and natural killer (NK) cells) to the tumor site might be involved in the vaccinia virus (VACV)-mediated immune response in different canine cancer xenograft models [69,70]. The presence of such activated inflammatory cells in the tumor tissue may enhance the antitumor effect by increasing the phagocytic or cytotoxic activities of these cells 71,72. In addition, an increase in proinflammatory interferon-gamma (IFN-gamma), interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), interferon gamma-induced protein 10 (IP-10), macrophage inflammatory protein-1 alpha (MIP-1 alpha), macrophage inflammatory protein-1 beta (MIP-1 beta), monocyte chemotactic protein-1 (MCP-1), and monocyte chemotactic protein-3 (MCP-3) was observed in vaccinia virus-infected canine xenografted mice [73]. Many of these proteins stimulate innate immunity mediated by dendritic cells, neutrophils, macrophages and NK cells. OVs naturally prevent neoangiogenesis either by direct infection and destruction of tumor vasculature [74] or “vascular normalization” in tumor tissue, as described by Winker and colleagues [75]. Additionally, oncolytic viruses can be armed to enhance their natural antiangiogenic ability. Vascular Endothelial Growth Factor (VEGF) is a key regulator of tumor angiogenesis and several anti-VEGF strategies have been developed for the treatment of different cancers [76,77]. Vaccinia virus inhibits the tumor growth in canine and feline xenografts by expressing anti-VEGF antibodies which significantly decreased neoangiogenesis at the tumor site [78,79]. Therefore, strategies employing virus-encoded and delivered anti-VEGF antibodies in combination with OV may be effective therapeutic approaches for pet cancer patients. For instance, we could envision administering OVs first by a series of intravenous infusions to ensure maximum distribution of the OV to all metastatic sites followed by multiple intratumoral in situ vaccine boosts. This type of administration regimen has already been piloted with an oncolytic vaccine [80]. The use of
recombinant OVs as clinical biotherapies, it is important to determine whether viremia could be induced that could result in shedding of the OV. The use of Ad5-prime/MG1-booster vaccination as a promising, novel therapy for testing in the context of veterinary clinical trials [81-83].

Figure 3 Possible mechanisms of oncolytic virus-mediated tumor ablation [68].

MΦ: Macrophages; NK: Natural Killer Cells; DC: Dendritic Cells; Neutr: Neutrophils; IFN-gamma: Interferon-gamma; IL-2: Interleukin-2; IL-6: Interleukin-6; TNF-alpha: Tumor Necrosis Factor Alpha: IP-10: Interferon Gamma-Induced Protein 10; MIP-1 Alpha: Macrophage Inflammatory Protein-1; MCP-1: Monocyte Chemotactic Protein-1; MCP-3: Monocyte Chemotactic Protein-3; TAA: Tumor-Associated Antigens

Limitations and prospects of cancer gene therapy

Despite certain hopeful outcomes in cancer gene therapy, there are many restrictions to overcome [78,79]. Only a restricted number of therapeutic genes can be used in clinical trials. Vectors are not efficient in vivo. Although the high transfection efficiency with adenovirus in vitro is well documented, it is still not clear whether adenoviral vectors are effective in vivo in solid tumor models [79].

Table 1 Advantages and disadvantages of oncolytic viruses for cancer therapy.

<table>
<thead>
<tr>
<th>Virus species</th>
<th>Virus strain</th>
<th>Genetic/synthetic modifications</th>
<th>Tumor selectivity</th>
<th>Immune function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Ad5/Ad3 or Ad5/Ad35</td>
<td>Engineered expression of fiber-knob proteins</td>
<td>Virus binds to tumor cells; avoids sequestration in liver Cells</td>
<td>Limited tumor infection</td>
<td>Ad as a single agent produces poor antitumor immunity; combined use of immunomodulatory</td>
</tr>
</tbody>
</table>

Figure 4 Infection and killing of tumor cells by an oncolytic virus [47]. a. Viruses interact with specific cell-surface receptors. As these proteins are overexpressed by tumor cells (blue) compared with normal cells (orange), the virus will probably infect the tumor cell. b. Following binding to the cell surface receptor, the virus is internalized by endocytosis or membrane fusion, and its genome is released into the cell. Depending on the type of virus, replication and viral gene expression 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, the virus is largely dependent on cellular machinery for viral gene expression and synthesis of viral proteins. Viral gene expression and replication leads to the activation of cellular antiviral defences, such as apoptosis, that are operational in normal cells but are often inactivated in tumor cells. Expression of viral proteins will eventually lead to immune-mediated lysis of infected cells 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. Alternatively, cells might be lysed owing to 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. TCR, T-cell receptors.
Pseudo-typed viruses (e.g., Ad5 delta-24R GD) CAR/integrin-binding deleted Ad, added receptor tumor-targeting ligand Enhanced tumor specificity Attenuated viral spread agents enhances antitumor effect

Coating with polyethylene glycol or other polymers; encapsulation with liposomes Avoid immune detection and viral clearance in the bloodstream Minimal inherent tumor selectivity; mild virus related infection Viral-mediated immunogenic cytotoxicity Potential for antiviral immunity [87-90]

JX-594 TK-deletion GM-CSF (+) Productive replication in tumor cells; enhanced viral spread Mild virus-related infection Enhanced tumor infiltration of eosinophils, APC and CTLs Stimulates potent cellular and humoral immune response to transgene

CDV Natural tumor tropism as cellular receptor for entry CD46 is expressed on tumor cells. Only lymphoma therapy data [53]

Replicates only in cells with E1B-19K deletion (i.e., anti-apoptotic factor) Latent infection is possible with native virus Co-treatment of virus with cyclophosphamide to abrogate host antiviral immunity Potential suppression of oncolytic virus-mediated antitumor immunity [91-93]

CVA13, CVA15, CVA18, CVA21, CVB3 Inherently tumor-selective strain Cancer-selective receptors ICAM-1, DAF, and CAR Potential or systemic toxicity Native virus induces strong immunity exposure Many patients have prior antiviral immunity [94]

Reovirus Nonengineered Inherently tumor-selective species, only replicates in cells with activated Ras-pathway and defective PKR Potential or mild toxicity Antigenicity generates immune response Antitumor response can be enhanced with chemotherapies Potential for antiviral immunity [95,96]

Conclusion

The substantial prevalence and death associated with cancers continue to challenge modern medicine to develop more reliable therapies. One of the greatest hopeful novels of cancer therapies is oncolytic virotherapy. This process is based on the capability of OVs to infect and lyse tumor cells and to initiate tumor-specific immunity. Oncolytic viruses including human and canine adenoviruses, canine distemper virus (CDV), reovirus and vaccinia virus strains have been tested with substantial results in preclinical studies. As in human, the most important challenges of oncolytic virotherapy for the successful clinical use of OVs in veterinary practice are a reduction of viral toxicity, optimization of virus delivery to the tumor, and enhancement of viral spread throughout the tumor mass. Recently, the first clinical studies with vaccinia and adenovirus for canine cancer therapy are underway and we look forward to the forthcoming demonstrations of clinical utility.

Declarations

Availability of data and materials

The findings were declared from available data source. All possible required information are attached and included in the manuscript. Moreover, raw data is available in the hand of the corresponding author. All coauthors gave full responsibility for corresponding author to share or discuss with editors and reviewers in review process.

Competing interests

The authors have no conflict of interests to declare.

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