

## Cancer-selective Mechanisms of Oncolytic Viruses

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### Abstract

Viruses that can be used for the treatment of cancer are referred to as oncolytic viruses. These viruses selectively infect cancer cells and induce cell death through a process of viral replication, proliferation, and budding. Diverse oncolytic viruses exist, and the genetic mechanisms of their cancer-selective killing strategies vary with the genetic diversity of each virus. Therefore, accurate information on viral genes and an understanding of cancer-selective killing mechanisms are required to study the treatment of cancer using oncolytic viruses. These require the storage and utilization of large amounts of data related to oncolytic viruses and cancer and studies based on enormous data sets using computer-based bioinformatics approaches. Such resources will aid in the development of novel cancer therapeutics using oncolytic viruses by providing the scientific basis for problem solving and decision making. In this study, we discuss the anti-cancer mechanisms of oncolytic viruses, classify them into four categories, and investigate the anti-cancer activities of different viruses. In addition, we evaluate the need for interdisciplinary research on oncolytic viruses by considering future research and therapy directions based on our understanding of molecular mechanisms, genetic mechanisms, and research achievements.

**keywords:** *Oncolytic virus, cancer-selective mechanism, oncolysis, oncolytic virotherapy, immunotherapy*

### Introduction

Cancer is a major cause of death worldwide and should be resolved as soon as possible to improve human health and well-being. The conventional cancer treatment methods include surgical intervention, chemotherapy, and radiation therapy. In recent years, new treatments such as immunotherapy and gene therapy have also been used [1]. Studies on cancer treatment methods using viruses have also been performed [2]. Viruses that can be used for cancer treatment are referred to as oncolytic viruses, viruses that kill cancer cells. These viruses selectively infect tumor tissue and cause apoptosis through viral replication and proliferation within cancer cells. They show potential as novel cancer therapeutics by selectively killing cancer cells while causing no harm to normal tissues [3]. Oncolytic viruses can be divided into viruses that have selective infectivity towards

cancer cells and those that can kill cancer cells through artificial manipulations and modifications geared toward the acquisition of selective infectivity. Viruses such as coxsackievirus, echovirus, reovirus, measles virus, poliovirus, and myxoma virus are included in the former group, and other viruses such as adenovirus, vesicular stomatitis virus, influenza virus, measles virus, vaccinia virus, and Newcastle disease virus belong to the latter group [4]. These cancer-killing viruses can be divided into DNA viruses and RNA viruses, and can function as 'cancer killers' through various anti-cancer mechanisms. Research on the development of cancer therapeutics with viruses is ongoing, and the anti-cancer effects of these virus-based therapeutic agents are being explored. Approximately 40 types of oncolytic viruses are being studied for their use as therapeutic agents [5]. Clinical trials of oncolytic viruses targeting various types of cancer are currently underway, and their

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anti-cancer effects are being demonstrated. Certain viruses are more commonly used due to their modification through genetic engineering.

Oncolytic viruses show diverse and complicated genetic mechanisms of killing cancer cells [6]. Table 1 shows features of major oncolytic viruses and their anticancer mechanisms. The diverse anti-cancer mechanisms of oncolytic viruses suggest that viruses can be applied as cancer therapeutic agents. However, our understanding of these mechanisms remains limited due to the lack of genetic evidence for the various mechanisms of cancer cell death by these viruses [7]. In recent years, studies have been performed to increase the efficiency of viral oncolysis and on genetic engineering methods to alter the cancer selectivity of viruses, and numerous studies have explored the design of novel oncolytic viruses. To construct oncolytic viruses that are safe and effective therapeutic agents, it is important to increase their specificity towards cancer cells, maximize their efficiency, and assess their safety [8]. Initially, an accurate understanding of the oncolysis strategies of each oncolytic virus is required. Studies of new mechanisms, the genes and proteins of the viruses and host cells involved in anticancer mechanisms, and characteristics of novel oncolytic virus candidates and genetic engineered viruses will be performed by studying the cancer-killing mechanisms used by each virus.

### **Cancer-selective infection: targeting cancer cell surface antigens**

Although all oncolytic viruses have the ability to selectively kill cancer cells, different viruses employ different mechanisms. Figure 1 shows various strategies of oncolytic viruses that cause cancer-selective infectivity. In this article, we explore these mechanisms by classifying them into four categories and investigate the anti-cancer viral activities of each mechanism. We discuss the necessity of interdisciplinary research on oncolytic viruses as effective cancer therapeutics, considering future research directions.

Viruses must first undergo attachment and penetration through cellular surface antigens to infect a host cell. Initially, the surface antigen protein of the virus attaches to specific receptor

proteins on the surface of target host cells. The entrance of a virus into a host cell and transmission of the viral genome into its cytoplasm occurs through interactions between proteins of the virus and host cell. The virus enters the cell through viral membrane fusion in the case of enveloped viruses and by endocytosis in the case of non-enveloped viruses [9]. Viral attachment events during the infection process are the first step in the viral life cycle. This step is responsible for determining the host range of viruses due to the large variety of host cell surface proteins [10]. Therefore, cancer cells expressing a specific surface antigen may be targeted by specific viruses.

Several viruses, including mumps virus, reovirus, Newcastle disease virus, measles virus, and coxsackievirus, exhibit selective infectivity toward cancer cells, and other viruses, including adenovirus, vesicular stomatitis virus (VSV), herpes simplex virus (HSV), and vaccinia virus, can show selective infectivity towards cancer cells after genetic manipulation and transformation [8]. The first studied virus that showed potential as an oncolytic virus was an adenovirus [11]. Adenoviruses are enveloped DNA viruses in which the capsid protein surrounding the DNA genome contains 'fibre' protein. Because fibre protein can use coxsackie and adenovirus receptor (CAR) as a receptor protein, an adenovirus must be modified to create cancer-selective infectivity since CAR is expressed in both normal cells and cancer cells [12]. Measles virus has inherent cancer-selective infectivity, and can be genetically modified to show increased specificity towards cancer cells. Hemagglutinin (H) protein, an attachment protein present on the envelope of measles virus, can use CD46 as a receptor protein. Since CD46 is expressed on various cancer cells, the measles virus has a higher specific infectivity for cancer cells [13, 14]. In addition, many viruses show high infectivity toward cancer cells, including coxsackievirus, poliovirus, echovirus, and VSV [3, 15]. Thus, various viruses can exhibit specificity for cancer cells, obtain anticancer mechanisms through genetic manipulations, or exhibit a high infectivity rate toward cancer cells depending on the type of receptor protein used. Oncolytic viruses that are capable of targeting various cancer cells are being developed and targeted towards clinical trials.

**Table 1.** Features of major oncolytic viruses and their anticancer mechanisms

Virus	Genome	Therapeutic(s)	Mechanisms
<b>Targeting of cancer-specific surface antigen (natural)</b>			
Coxsackievirus	ssRNA(+)	CAVATAK	· Targeting DAF / intercellular-adhesion molecule 1 overexpressed in malignant melanoma cells
Poliovirus	ssRNA(+)	PV1(RIPO)	· Targeting CD155 overexpressed in brain glioma cells
Echovirus	ssRNA(+)	-	· Targeting integrin $\alpha_1\beta_2$ overexpressed in ovarian cancer cells
<b>Targeting of cancer-specific surface antigen (artificial)</b>			
Adenovirus	dsDNA	-	· Connecting a bispecific antibody to the fibre protein to bind to epidermal growth-factor receptor (EGFR), which is highly expressed in tumor cells
Measles virus	ssRNA(-)	MV-CEA MV-EGFR MV-CD38	· Modification to recognize cancer-specific antigens such as CD38 and carcinoembryonic antigen (CEA), but not CD46 and SLAM, which are receptors for the H protein
Vesicular stomatitis virus	ssRNA(-)	-	· Modification to enable the production of a single-chain antibody that binds the Sindbis glycoprotein to be bound to HER2 / NEU
<b>Selective replication in cancer cells</b>			
Reovirus	dsRNA	Reolysin	· High replication rate in cancer cells that have abnormal persistent activity of the RAS pathway
Vaccinia virus	dsDNA	-	· High replication rate in cancer cells that have abnormal activity of EGFR, which is related to the RAS pathway
Vesicular stomatitis virus	ssRNA(-)	VSV-IFN- $\beta$	· Increase in the replication rate of cancer cells showing defects or downregulation of the interferon pathway
Adenovirus	dsDNA	Onyx-015	· Genetic modification for replication and proliferation exclusively in cancer cells with abnormalities in the p53-mediated cell cycle (deletion of the E1 gene)
Herpes simplex virus	dsDNA	NV1020	· Genetic modification for specific infection of cancer cells whose suppressive function of viral replication and proliferation in cells is not expressed properly (deletion of the E1B gene)
Influenza virus	ssRNA(-)	delNS1	· Induction of selective replication in cancer cells with inhibited PKR (deletion of the NS1 protein)
<b>Targeting the cancer microenvironment</b>			
Reovirus	dsRNA	-	· Acquisition of pathogenicity within a cancer microenvironment that is overexpressing proteases
Measles virus	ssRNA(-)	-	· Enhanced infectivity of cancer cells by replacing the furin site of the F protein with an MMP2 cleavage site
Newcastle disease virus	ssRNA(-)	-	· Enhanced infectivity of cancer cells by replacing the furin site of the F protein with an MMP2 cleavage site
<b>Application to immunotherapy</b>			
Herpes simplex virus-1	dsDNA	OncoVEX	· Used for immunotherapy with a live attenuated version created by genetic modification to delete ICP34.5 and the $\alpha 47$ gene and insert GM-CSF
Vaccinia virus	dsDNA	GLV-h68	· Modified to express GM-CSF
Adenovirus	dsDNA	-	· Expression of the cytokine IL-12, the chemokine RANTES, and the antimicrobial peptide $\beta$ -defensin
Vesicular stomatitis virus	ssRNA(-)	VSV-hIFN $\beta$	· Expression of IFN- $\beta$

Representative oncolytic viruses, their genome types, the name of developed cancer therapeutics using oncolytic viruses, and the respective cancer-selective killing mechanisms are described.

### Cancer-selective replication

Systematic infection by viruses requires viral genome replication using the replication machinery of host cells [16, 17]. Understanding the mechanisms that occur in cancer cells and the characteristics of the associated cancer micro-environment are needed to determine why these processes occur selectively in cancer cells and not in normal cells [18].

Several viruses show cancer-specific replication, including reovirus, which replicates exclusively in cancer cells [19]. Reovirus, an enveloped dsRNA virus, shows selective infectivity toward and a high replication rate in cancer cells, along with abnormal activity of the RAS pathway. Reolysin, which was developed using reovirus, has shown potential as a novel cancer therapeutic in clinical trials [20]. Vaccinia virus also has a high replication rate in cancer cells with abnormal epidermal growth factor receptor (EGFR) activity, which is relevant to the RAS pathway. VSV has a high replication rate in cancer cells due to their lower antiviral responses than normal cells. Defects or downregulation in the interferon pathway in cancer cells allow increased specificity of viruses towards cancer cells [21].

In addition to viruses with inherent cancer-selective replication, some viruses can obtain anticancer mechanisms through genetic engineering. Adenovirus can be used as an oncolytic virus after genetic modification; the modified version replicates and proliferates only in cancer cells with an abnormal p53-mediated cell cycle. The adenovirus E1B gene has been found to inactivate the tumor suppressor protein p53, so that adenovirus with a deleted E1 gene has a relatively high replication rate in cancer cells with deficient p53 because it no longer expresses E1B protein [22]. HSV with a deleted E1B gene selectively infects cancer cells; because the suppression of viral replication and proliferation do not work properly due to this deletion, apoptosis is induced [23]. Influenza virus infects pigs, birds, and humans, causing respiratory diseases. In addition, it is capable of being utilized as an oncolytic virus to perform a specific replication and multiplication in cancer cells. The non-structural protein 1 (NS1) protein produced by the influenza virus plays an important role in its pathogenicity by inhibiting

PKR-mediated antiviral responses. Therefore, cancer-specific apoptosis can be induced after viral replication in normal cells with normal PKR activity, and selective viral replication can occur in cancer cells by deleting the NS1 protein from viruses [24].

In summary, genetic engineering techniques have been used to create numerous viruses that show cancer-selective replication and proliferation as well as improved anti-cancer effectiveness. New mechanisms of cancer-specific replication have been identified, likely with more to come. Studies to explore various viruses as cancer therapeutics based on these mechanisms will serve as foundations for novel cancer therapeutics that utilize oncolytic viruses.

### Targeting cancer microenvironment

Differences exist between the microenvironments of various cancer cells, and viruses targeting the cancer microenvironment can be used for cancer-specific killing.

Reovirus, a non-enveloped virus that is sensitive to cancer microenvironments, typically infects cells of digestive organs. Proteases are present at high levels in digestive organs and can convert non-infectious reovirus into infectious viral particles. Proteases are commonly overexpressed in the cancer microenvironment, and reovirus can act as an infectious particle by activating a protease. For this reason, reovirus has a higher infection rate of cancer cells than of normal cells, and eventually causes cancer-selective apoptosis [25].

Use of the cancer microenvironment to increase selectivity towards cancer cells can also be applied to measles virus and Newcastle disease virus. The fusion (F) protein of measles virus mediates the fusion of viruses with the cell membrane; it is a surface protein that functions during virus attachment and penetration into the host cell. F protein typically exists as an inactive precursor, and the fusion peptide is exposed when cleaved by furin. Therefore, it is possible to construct a virus that is converted to its active form only in the protease-rich cancer microenvironment by altering the F protein [26]. Proteases such as matrix metallo-proteinase 2 (MMP2) are expressed at high levels

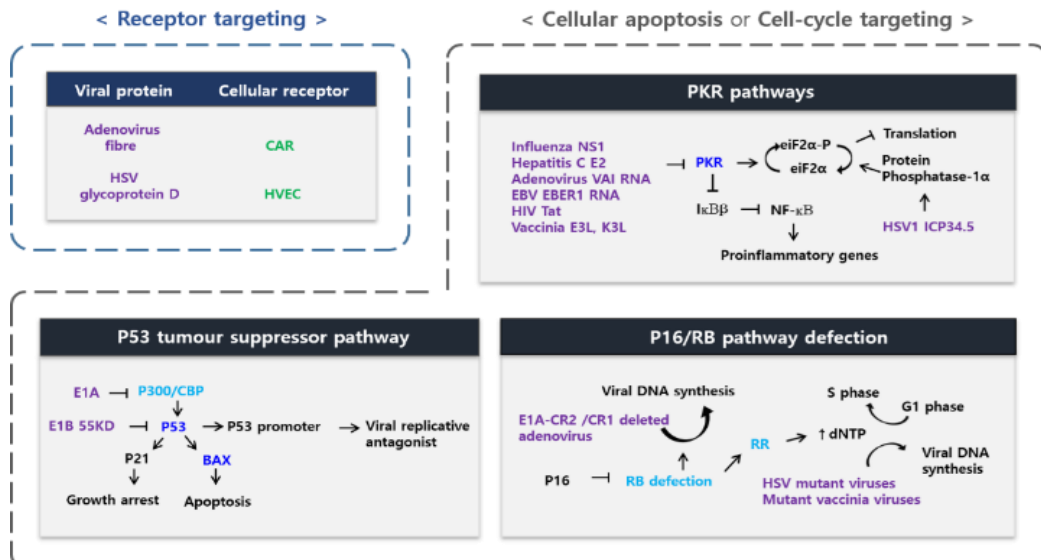
in various types of cancer cells. Therefore, the infection rate of measles virus towards cancer cells can be increased by activating viral pathogenicity in the vicinity of cancer cells through the modification of the F protein to contain an MMP2 cleavage site in place of the furin site. Because Newcastle disease virus also contains the F protein, it is possible to increase the viral infection rate by targeting the cancer microenvironment in the same way as the measles virus [26]. To target viruses to the cancer microenvironment using modification and replacement of the cleavage site of the viral fusion protein, studies on how to increase the specificity of viruses toward cancer cells or the cancer microenvironment are required.

### Immunotherapy using oncolytic viruses

Conventional anticancer therapies typically include surgical intervention, radiation therapy, or chemotherapy, depending on the condition of individual patients. In addition to these traditional therapies, studies on immunotherapy using the patient's immune system and the introduction of genes involved in cancer-related mechanisms are currently underway, and the results of several

recent clinical trials have been successful [1, 2].

Cancer therapy using oncolytic viruses is a novel cancer treatment method. The cancer-selective killing mechanisms of certain viruses, as well as the results of substitution, introduction, or deletion of viral genes through genetic modification, suggest that viral gene therapies can be used for cancer treatment. Gene therapy using oncolytic viruses does not have as high of cure rate as conventional anticancer therapies. However, further studies are warranted because this type of gene therapy causes fewer side effects than conventional chemotherapy. Anticancer immune therapy shows efficacy with remarkably low side effects because the individual patient's own cells are used for the treatment, unlike conventional anti-cancer agents. Immune cells such as natural killer (NK) cells, dendritic cells, B cells, and T cells are used in cancer immunotherapy and can be combined with existing anticancer therapeutic agents [27]. Oncolytic viruses can also be used for anticancer immunotherapy. It is possible to induce apoptosis in cancer cells indirectly by stimulating anticancer immune responses through oncolytic viruses [28]. Oncolytic viruses used in cancer immunotherapy function as vectors for the expression of genes that stimulate viral-mediated immune responses, and



**Figure 1. Various strategies for cancer-selective infectivity of oncolytic viruses.** Oncolytic viruses that can selectively kill cancer cells have various strategies for targeting cancer cells due to different genetic and biomolecular characteristics. As a result of these strategies, various viral mechanisms for cancer-selective killing are used by each virus.

viruses such as herpes simplex virus-1 (HSV-1), vaccinia virus, and adenovirus have these anticancer mechanisms [29]. HSV-1 can be used in anticancer immunotherapy after genetic manipulation. OncoVEX GM-CSF, an attenuated HSV-1 created by genetic modification, has been evaluated in clinical trials in patients with metastatic melanoma [30]. The vaccinia virus line JX-594, which has been modified to express GM-CSF, was shown to have anticancer effects in patients with liver cancer and melanoma [31]. GM-CSF can also be introduced into adenovirus, resulting in anticancer effects. Such lines have been shown to stimulate the immune system against prostate cancer through experiments in hamster. It is difficult to predict the responses and effects of anticancer immune therapy with oncolytic viruses, and extensive examination of appropriate models is needed. Therefore, studies on the molecular biology of the virus and immune system of the host cell are required.

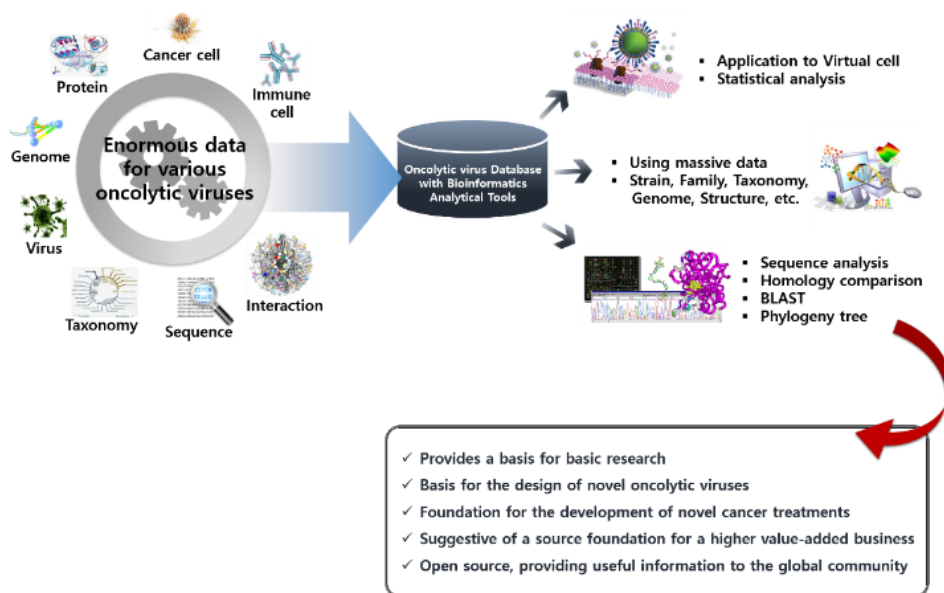
### Conclusion

The development and use of various cancer treatments require the genetic analysis of oncolytic viruses with potential as advanced cancer therapeutics and studies on their anticancer mechanisms. Mouse models, cancer cell lines, and clinical experiments in cancer patients have been used in most studies on oncolytic viruses. However, these studies are time-consuming and costly, and can have safety risks due to the biological characteristics of the viruses. Thus, novel methods to address these problems are required. Bioinformatics studies will provide a novel perspective in oncolytic virus studies. Above all, understanding the complex mechanisms of the various viruses is required to study their complex cancer-selective mechanisms. This will require the construction of databases and analytical tools that enable researchers to utilize viral sequence information and other biological data. Figure 2 shows the utilization and expected achievements of database-based bioinformatics approaches in studies of oncolytic viruses.

Correlation analysis of genetic properties and the anticancer mechanisms of each virus will be studied using integrated databases, and candidate viruses

for clinical trials will be evaluated using systematic analysis of oncolytic viruses based on studies of genetic information and biomolecular mechanisms. Studies of the anti-cancer mechanisms of oncolytic viruses will benefit from the effects of enhanced competitiveness of national medical technology by contributing to the development of novel cancer therapeutics through the analysis of genetic variables in viruses and host cells. From an international perspective, studies on cancer have great value to society in that they have high value if state-of-the-art therapeutics are made available, with global demand. More diverse data will be accumulated through future studies. It is believed that the convergence of interdisciplinary research and the application of findings to relevant studies will be accomplished by sharing information and promoting communication among researchers during the construction of an integrated database. Collaborative studies on oncolytic viruses performed in various academic fields, including bioinformatics, are expected to provide novel insights into the development of strategies for cancer treatment.

In this review, various cancer-selective oncolysis mechanisms of oncolytic viruses that have potential as cancer therapeutics were explored by classification according to individual viral genetic and molecular features, and the anti-cancer activities of each virus and research trends were investigated. In addition, the necessity of interdisciplinary research including bioinformatics was evaluated for studies on oncolytic viruses. Cancer is the leading cause of death in Korea, and studies on the development of novel cancer therapeutics are important for public health. Because social and economic losses have gradually increased due to a steady increase in the incidence of cancer and mortality, novel cancer therapeutics are required. It is believed that bioinformatics approaches capable of scientific analysis based on the integration and processing of a large amount of data will provide novel solutions to replace conventional research methods. Newly derived research results based on interdisciplinary studies including bioinformatics approaches will allow for the creation of novel information and applications. As a result, the groundwork for future studies on cancer treatment is available and is expected to



**Figure 2. Use of bioinformatics for studies of oncolytic viruses.** An analysis of cancer-selective killing mechanisms of viruses having potential as novel cancer therapeutics by constructing a specialized database integrating enormous amounts of data for oncolytic viruses is shown. Based on the constructed database, the development of an integrated bioinformatics technique facilitating the analysis and prediction of biological mechanisms can be undertaken.

provide a scientific basis for the practical application of advanced cancer treatment methods.

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