

REVIEW

Oncolysis by paramyxoviruses: multiple mechanisms contribute to therapeutic efficiency

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Oncolytic paramyxoviruses include some strains of Measles, Mumps, Newcastle disease, and Sendai viruses. All these viruses are well equipped for promoting highly specific and efficient malignant cell death, which can be direct and/or immuno-mediated. A number of proteins that serve as natural receptors for oncolytic paramyxoviruses are frequently overexpressed in malignant cells. Therefore, the preferential interaction of paramyxoviruses with malignant cells rather than with normal cells is promoted. Due to specific genetic defects of cancer cells in the interferon (IFN) and apoptotic pathways, viral replication has the potential to be promoted specifically in tumors. Viral mediation of syncytium formation (a polykaryonic structure) promotes intratumoral paramyxovirus replication and spreading, without exposure to host neutralizing antibodies. So, two related processes: efficient intratumoral infection spread as well as the consequent mass malignant cell death, both are enhanced. In general, the paramyxoviruses elicit strong anticancer innate and adaptive immune responses by triggering multiple danger signals. The paramyxoviruses are powerful inducers of IFN and other immuno-stimulating cytokines. These viruses efficiently promote anticancer activity of natural killer cells, dendritic cells, and cytotoxic T lymphocytes. Moreover, a neuraminidase (sialidase), a component of the viral envelope of Newcastle Disease, Mumps, and Sendai viruses, can cleave sialic acids on the surface of malignant cells thereby unmasking cancer antigens and exposing them to the immune system. These multiple mechanisms contribute to therapeutic efficacy of oncolytic paramyxoviruses and are responsible for encouraging results in preclinical and clinical studies.

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INTRODUCTION

Metastatic cancer remains largely an incurable disease which requires development of novel therapeutic strategies. While the accumulating knowledge on the molecular basis of cancer offers new potential targets for anticancer drugs, there is an alternative approach that relies on mechanisms developed through the millions of years of human coexistence with viruses. The viruses emerge as promising instruments against cancer. Substantial selectivity of infection and replication in cancer cells is characteristic of many viruses, and their therapeutic efficiency and safety can be dramatically improved by genetic manipulations.

Paramyxoviruses represent a well-studied model of oncolytic viruses with promising therapeutic potentials. The antitumor efficacy by oncolytic paramyxoviruses appears to be associated with three properties of the viruses: (i) selective replication in tumor cells, (ii) oncolytic properties by the viruses *per se*; and (iii) immunostimulatory capacity of the viruses. Studies on viral oncolysis mediated by paramyxoviruses have revealed some properties that collectively contribute to strong anticancer effects. Some of these properties are common to many oncolytic viruses and some are specific to oncolytic paramyxoviruses only. All these properties are described below.

BIOLOGY OF PARAMYXOVIRUSES

Paramyxoviruses (members of the *Paramyxoviridae* family) are associated with a number of diseases in animals and humans, such as measles virus (MV), mumps, and several respiratory infections. Sendai virus (SeV) affects mice and some other rodents. Newcastle disease virus (NDV) is associated with a contagious disease affecting many domestic and wild species of birds. SeV and NDV were not found to be associated with any serious human diseases, while MV and mumps are well-studied human pathogens. Few representatives of paramyxoviruses NDV, SeV as well as attenuated vaccine strains of MV and mumps viruses were tested as oncolytic agents in multiple model experiments and in a few clinical trials.

Paramyxoviruses enter the life cycle with binding of their attachment protein to an appropriate cell-surface receptor. There could be either direct fusion of the envelope with plasma membrane¹ assisted by the F protein, which is activated by the interaction with sialic acid-containing surface glycoproteins,² or the virus can enter the cell through the endocytic route with fusion occurring in acidic conditions inside endosomes.³ As a result, the nucleocapsid containing viral genome is released into the cytosol where viral replication takes place.

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Viral RNA-dependent RNA polymerase transcribes the genes into mRNAs, which are then translated into structural and nonstructural proteins. The transcription starts from a single promoter located at the 3' end of the genome and then may either terminate within specified regions between each viral gene or proceed further downstream. This mode of transcription is responsible for the observed product polarity in which the genes closest to the 3' end of the genome are expressed more abundantly than their downstream counterparts.¹

This mechanism is a simple and effective way for transcription level regulation to generate the necessary balance of viral products. A concentration of the most abundantly synthesized nucleoprotein determines the time at which RNA-dependent RNA polymerase switches from gene transcription to genome replication. Replication involves synthesis of full-length positive-strand RNAs, which are then transcribed into the progeny genomic minus-strand RNAs. The maturing virions finally gain their envelopes with the membrane-trapped viral glycoproteins by budding through the outer membrane. New virions can then infect other cells and enter new life cycles. An alternative pathway for spreading viral infection involves fusion of infected cells with their neighbors and formation of syncytia.⁴ Viral fusion proteins used by the virus to enter the cell are exposed to the cell surface of infected cells inducing fusion with plasma membranes of neighboring cells. Therefore, a single virion can potentially infect and kill dozens of cells.

THREE LEVELS OF CANCER-SPECIFIC INFECTION AND SPREADING FOR PARAMYXOVIRUSES

Receptors for paramyxoviruses are frequently overexpressed in malignant cells

A first level of specificity for cancer cells is related to overexpression of specific receptors for oncolytic viruses (Figure 1). NDV, mumps, and SeV use sialic acid-containing sialoglycoproteins as the cell surface receptors.^{5,6} The abundant presence of sialoglycoproteins on the surface of cancer cells^{7,8} most likely promotes preferential association of the virus with malignant rather than with normal cells and contributes to their selective cytolytic effect in primary tumors and in metastases. Perhaps high sensitivity to SeV mediated cell death of sialic acid-rich prostate carcinoma cells, in comparison with normal prostate epithelium, is explained by SeV's preferential association with malignant cells.⁹

The attenuated measles virus (Edmonston strain) (MVES) uses the CD46 receptor, which is a regulator of complement activation that is universally expressed in all nucleated human cells but is often overexpressed in tumor cells.¹⁰ MVES can kill cells that overexpress this receptor without significant cytopathic effect against nontransformed cells expressing low receptor levels.¹¹ Nectin 4 has recently been identified as the additional receptor for MV.¹² It is a member of

adhesion receptors of the immunoglobulin superfamily localized to the adherents' junctions of epithelial cells. Nectin 4 can be considered as a tumor cell marker for breast, lung, and ovarian cancers,¹³⁻¹⁵ suggesting that it can be partially responsible for the selectivity of measles virus toward cancer cells. Table 1 summarizes information related to viral receptors and their overexpression in malignant cells.

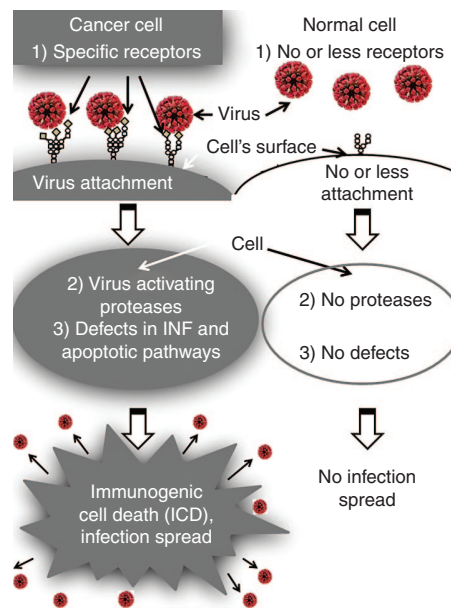


Figure 1 Three levels of cancer-specific infection spread for paramyxoviruses. (1) First level of virus specificity for cancer cells is related to overexpression of specific receptors for paramyxoviruses. Sialic acid residues in a form of sialoglycoproteins serve as receptors for NDV, mumps, and SeV. These sialoglycoproteins are frequently overexpressed in malignant cells. CD46 and nectin 4 serve as natural receptors for attenuated strain of MV, and they are also frequently overexpressed in malignant cells. (2) Second level of virus specificity is related to cancer cells' specific proteases. The replication cycles of paramyxoviruses require protease cleavage of viral glycoproteins for productive cell enter. Desirable proteases for oncolytic viruses that can activate them are expressed preferentially by cancer cells. For example, matrix metalloproteinases are overexpressed in almost all human cancer cells. (3) Third level of virus specificity is related to cancer cells' specific genetic defects that allow viral replication. During the process of malignization, a cancer cell accumulates many genetic changes. Along with mutations that promote accelerated proliferation and invasion, many cancerous cells are losing abilities to produce interferon (IFN) and to respond to IFN by apoptotic pathway induction. Such abnormalities make these cells highly susceptible to viral infection. So, oncolytic paramyxoviruses due to abundance of cancer-specific receptors and due to cancer-specific genetic defects are more likely spreading among malignant cells rather than among normal cells.

Table 1 Oncolytic paramyxoviruses and their receptors overexpression in cancer cells

Virus	Receptor	Receptor overexpression
Newcastle disease virus (NDV)	Syalic acid containing molecules ²	Invasive and metastatic cancers ⁸
Sendai virus (SeV)		
Mumps		
Measles virus Edmonston strain (MVES)	CD46 (ref. 11)	Breast and ovarian cancers and other malignancies ^{157,158}
	Nectin 4 (refs. 12,159)	Breast, lung, and ovarian cancers ^{13,14,160}

Virus activation through cancer-specific proteases

A second level of cancer cells' specificity is related to cancer cells' specific proteases (Figure 1). The replication cycles of enveloped viruses, such as paramyxoviruses, require protease cleavage of viral glycoproteins for productive cell entry.¹⁶ Desirable proteases in cancer cells for oncolytic viruses should be expressed mainly by cancer cells. Such proteases exist; they are represented by matrix metalloproteinases.¹⁷ These molecules are endopeptidases that are overexpressed in almost all human cancer cells.^{18–20} It is still unknown whether these proteases can activate wild-type viruses. However, a recombinant Sendai virus,²¹ as well as MV constructs^{22,23} that are selectively activated in human tumor cells expressing matrix metalloproteinases, has been created.

Genetic defects of cancer cells

A third level of cancer cells' specificity is related to their specific genetic defects which allow viral replication (Figure 1). During the process of malignization, a cancer cell accumulates many genetic changes. Along with mutations that promote accelerated proliferation and invasion, many cancerous cells lose their ability to produce and respond to interferon (IFN) by apoptotic pathway induction.²⁴ Such abnormalities make these cells highly susceptible to viral infection.²⁵ So, due to the abundance of cancer-specific receptors, cancer-specific genetic defects, and proteases, oncolytic paramyxoviruses are more likely going to spread among malignant than among normal cells.

ONCOLYSIS MEDIATED BY DIRECT CANCER CELL KILLING THROUGH FORMATION OF SYNCYTIUM

Paramyxoviruses have a mechanism of virus infection spread without the release of mature virus particles from the cells. The mechanism includes fusion of infected cells with their noninfected neighbors. The fusion results in the formation of a syncytium, a large polykaryonic conglomerate originating from many cells. A single infected cell could trigger up to 50–100 neighboring cells to fuse together and to form a syncytium.²⁶ The process of cell fusion allows the virus to minimize exposure to host neutralizing antibodies. Usually, the syncytia survive no longer than 4–5 days.

Syncytium death is associated with nuclear fusion, premature chromosome condensation, and autophagic degeneration, accompanied by release of vesicles reminiscent of exosomes (syncytiosomes). "Dying syncytia produce significantly more syncytiosomes than normal cells or cells killed by irradiation, freeze thaw, or osmotic shock. These syncytiosomes also load dendritic cells (DCs) for antigen presentation more effectively than exosomes from cells dying by other mechanisms."²⁷

Viral fusogenic membrane glycoprotein expression causes syncytia development and consequent cellular immunogenic death,^{4,28} which can contribute to the efficiency of viral oncolysis. Consequently, the ability of viruses to induce the formation of syncytia might correlate with their oncolytic potential. This hypothesis is supported by some experimental evidence. The fusion protein of NDV was introduced into an oncolytic strain of vesicular stomatitis virus (VSV), with significant enhancement of oncolytic activity of the virus against multifocal hepatocellular carcinoma in the livers of immunocompetent rats.²⁹ Similarly, the oncolytic activity of a strain of replication-competent herpes simplex virus (HSV) was significantly increased after the introduction of a hyperfusogenic glycoprotein from the gibbon ape leukemia virus.³⁰ The fusogenic potential could be further increased by the introduction of amino

acid substitutions. A novel NDV variant harboring an L289A substitution within the F gene possesses enhanced fusion and oncolytic activities against rat hepatocellular carcinoma cells, both *in vitro* and in immunocompetent rats.³¹ A mutant of SV5 paramyxovirus that harbors a glycine-to-alanine substitution in the fusion protein was hyperfusogenic and displayed an enhanced oncolytic activity.³² Remarkably, even plasmid vectors^{25,33,34} or replication-deficient viruses^{35–37} expressing the fusogenic membrane glycoproteins are capable of promoting significant tumor regression, suggesting that cell fusion and syncytia formation substantially contribute to the oncolytic activity of paramyxoviruses. So, ability to kill cancer cells directly through formation of syncytium is an important property of oncolytic paramyxoviruses (Figure 2a).

SUMMARY OF FACTORS THAT AFFECT VIRUS PRODUCTION LEVEL IN MALIGNANT CELL

It is noticeable from the sections presented above that the factors potentially influencing paramyxovirus production levels in cancer cells are virus and cancer cell specific. So, a paramyxovirus representative and a cancer cell have to complement each other to achieve effective virus-mediated tumor destruction. A particular virus receptor should be overexpressed in a particular malignant cell for efficient virus attachment. A malignant cell should have genetic defects in IFN and apoptotic pathways that allow viral replication in this cell. A malignant cell should overexpress a specific protease for efficient viral particle maturation. The F protein of the paramyxovirus utilized should have a suitable proteolytic cleavage site for the malignant cell-specific protease to enable virus processing and maturation. The viral F protein should be able to induce malignant cell fusion (syncytium formation) that allows paramyxoviruses to minimize exposure to host neutralizing antibodies and maximize yield of infectious particles. Through genetic engineering, it is possible to create viral constructs with higher affinity to malignant cells receptor (described below in the section Improving tumor-specific targeting), constructs with better proteolytic processing abilities by cancer cells specific matrix metalloproteinases and constructs with better cancer cell fusing activities (described in the section *Optimization of syncytia formation and F protein cleavage site*). However, it is very difficult to optimize all the factors in one genetic construct for most efficient malignant cells attachment, replication, and particles maturation but perhaps it will be accomplished in future.

ONCOLYSIS MEDIATED BY PARAMYXOVIRUS-INDUCED STIMULATION OF THE ANTITUMOR IMMUNE RESPONSE

In addition to the direct killing of infected cancer cells, viral infection elicits systemic responses that strongly contribute to viral oncolysis. These responses include stimulation of antiviral innate immunity, such as the production of IFNs, other cytokines, and the activation of natural killer (NK) cells. Also, viral infection elicits adaptive immune responses that not only act to contain the infection by targeting the released viral particles and the infected cells, but also assist in the exposure of cancer cells to the antigen-presenting cells leading to activation of the anticancer immune response (Figure 2b).

Enhancement of cancer cell immunogenicity

Paramyxoviruses, including oncolytic paramyxoviruses, often modulate genes related to antigen presentation and enhance the immunogenicity of cancer cells. For example, it has been known that NDV upregulates human lymphocyte antigens (HLA) class I and

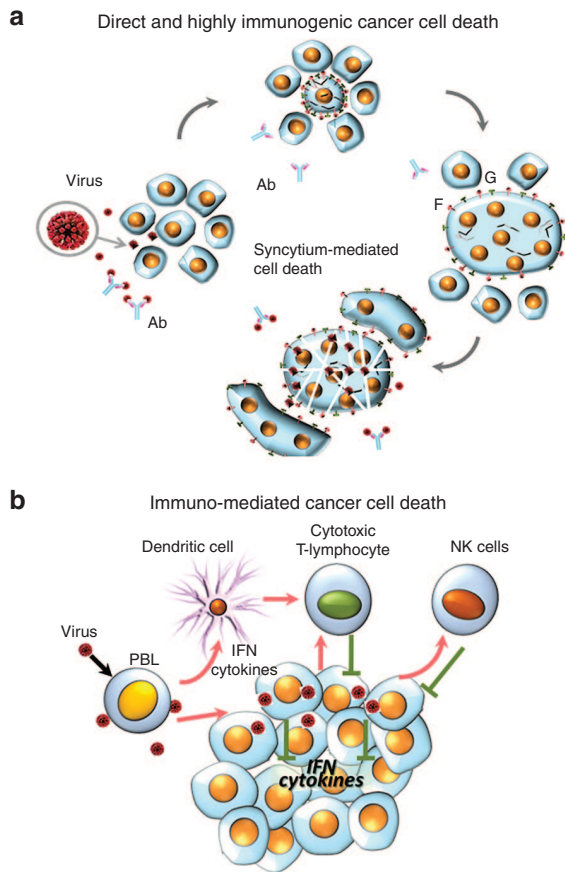


Figure 2. Direct or immuno-mediated cancer cells death. **(a)** Direct and highly immunogenic cancer cell death through formation of syncytia. Virus-infected cells start expressing viral fusion protein (F) at their cell surface that forces fusion of infected and surrounding noninfected cells and formation of large polykaryonic structures known as syncytia. The syncytia support continuous viral replication and further fusion with new uninfected cells. Eventually, the syncytia die. The syncytia formation allows viral infection to spread even in the presence of high titers of neutralizing antiviral antibodies. This type of cell death assists in viral oncolysis. Proteolytic cleavage of viral protein F0 is needed to activate it and transform it into F1. Only F1 can promote cell fusion into syncytia. For some representatives of paramyxoviruses, F0 that is expressed in infected human cells is fusion incompetent because of the absence of proteolytic cleavage to activate F-protein (F0) and transform it to F1. Therefore, syncytia formation could not expand in tumor tissues, unless they express a protease that could perform appropriate proteolytic cleavage. **(b)** Immuno-mediated cancer cell death. Paramyxoviruses stimulate innate and adaptive immunity that targets cancer cells. Malignant cells are frequently unable to react to virus infection by producing INF. They are also frequently unable to respond to INF by apoptotic pathway induction. In response to virus infection, normal cells are able to produce INF. Notably, virus induced INF is synthesized by macrophages, dendritic, endothelial and tumor stromal cells. Moreover, in response to virus infection, tumor stromal cells also secrete numerous cytokines that elicit a strong antitumor reaction by attracting macrophages, monocytes, natural killer (NK) cells and other components of the innate immune system. In addition, IFNs and cytokines stimulate dendritic cells (DCs) to educate cytotoxic T-lymphocytes to target tumor cells.

II as well as costimulatory molecule intercellular adhesion molecule 1 in human cancer cells.³⁸

Contribution of danger signals

Oncolytic viruses could play a crucial role in tumor rejection by helping to overcome immune suppression mediated by the tumor

microenvironment. Perhaps, to some degree, anticancer immune activation can occur even without replication of a virus in a tumor mass. It is likely the antitumor immune response can be triggered even if the virus is injected into skin or intratumoral.³⁹ By its nature, the virus acts as a danger signal, which is able to recruit and activate professional antigen-presenting cells such as DCs. DCs act as part of both innate and adaptive immunity and can prime adaptive T cell immune responses against specific, tumor-associated antigens (TAAs).⁴⁰ Paramyxoviral fusogenic membrane glycoproteins promote syncytia formation that triggers particular efficient TAA presentation by DC.²⁷

In general, DC activation can be induced by endogenous and exogenous danger signals.⁴¹ In current terms, the endogenous signals are damage-associated molecular patterns (DAMPs) and exogenous danger signals are pathogen-associated molecular patterns (PAMPs) molecules. DAMPs and PAMPs act to initiate immune responses to infection, pathological processes, and therapeutic processes such as oncolytic virotherapy.^{42–44} Cancer cells can evade the immune system by conditioning their microenvironment until viruses overcome the immunosuppressive environment. They produce immunosuppressive cytokines and recruit immunosuppressive cells, such as T regulatory cells (T-regs), myeloid derived suppressor cells, and M2 macrophages to maintain immune tolerance within the tumor.⁴⁵ Acting on both cancer and immune cells, oncolytic paramyxoviruses elicit potent adaptive immunity against specific tumor antigens and thus against cancer cells. These viruses operate as immuno-adjuvants to overcome the immunosuppressive tumor microenvironment, as cell-surface exposed viral antigens and products of dying cells provide potent danger signals. As a result, immunogenic cell death (ICD) of cancer cells gets promoted. It may resemble apoptosis, necrosis, pyroptosis, or autophagic cell death.^{46,47} DAMPs include the calreticulin (ecto-CRT) and heat-shock proteins (HSP70, HSP90) exposed on the cell surface, high-mobility group box 1 (HMGB1) protein, ATP, and uric acid released from dying cells. This release happens during mid to late phases of apoptosis, necrosis, or autophagy.

Introduction of PAMPs to cancer cells through virus infection is related to the previously described processes known as artificial heterogenization⁴⁸ or xenogenization⁴⁹ meaning that pathogens increase cancer cells visibility for immune system. The danger signals activate DCs, which cross-present TAAs to cluster of differentiation (CD) 8⁺ T cells for potent antitumor immunity. By this mechanism, MV induces ICD in human melanoma.⁵⁰ Following ICD of cancer cells infected with MV vaccine, the apoptotic bodies are ingested by DCs, which mature and then produce high levels of IFN- α , and cross-present TAAs, leading to a generation of tumor-specific CD8⁺ cytotoxic T cells.^{51,52}

Role of autophagy

Autophagy is a well-regulated process that mediates degradation and recycling of cellular proteins, organelles, as well as pathogens.⁵³ Autophagy plays important roles in both innate and adaptive immunity.^{54,55} A number of paramyxoviruses, including MV,⁵⁶ NDV,⁵⁷ and SeV,⁵³ have been shown to induce autophagy in infected cancer or normal cells. As mentioned earlier, death of syncytia is associated with autophagy.⁵⁸ Interestingly, the engagement of CD46, a ubiquitous human surface receptor, which is able to bind several different pathogens including MV, is sufficient to induce autophagy.⁵⁹ Autophagy enhances tumor immunogenicity not only by releasing DAMPs but also by promoting an antigen cross-presentation from

cancer cells to DCs and then to T cells. Cross-presentation of TAAs by DCs is promoted by the nonapoptotic (most likely autophagic) cell death induced by paramyxoviral fusogenic membrane glycoproteins.²⁷

Inhibition of the IFN response in target cells

One of the most important defense mechanism a cell has against viral infection is induction of IFNs and IFN-inducible proteins, resulting in suppression of protein synthesis and establishment of an antiviral state. However, viruses have evolved diverse strategies to evade or antagonize the IFN antiviral response proteins. Paramyxoviruses encode and use a number of proteins, which facilitate escape from cellular defense by blocking IFN production or/and signaling. NDV and mumps viruses use V-protein,^{60,61} SeV uses a family of C-protein,⁶¹ V-protein,⁶² and MV uses V-protein,⁶³ P-protein,⁶⁴ and C-protein⁶⁵ to block the IFN pathway.

During a process of malignization, a tumor cell genetically changes and gets rid of functions that serve the needs of the whole organism. Along with mutations that promote accelerated proliferation and invasion, the IFNs signaling is often compromised through a homozygous loss of the chromosome 9p21 region, which encodes type I IFN genes.²⁴ In malignant cells, virus may not affect IFN signaling as this pathway is already broken. However, sometimes during the genetic engineering process, it is necessary to minimize the potential impact of viral construct on healthy cells; therefore, viral genes, which block the IFN pathway, are deleted or attenuated.⁶⁶

Stimulation of the IFN response in peripheral blood leukocytes

Paramyxoviruses are known to be strong inducers of IFNs in human peripheral blood leukocytes (HPBLs). This property that has been used in biotechnology: the Sendai virus was chosen from among many other viruses for industrial-scale production of IFN from human leukocytes.⁶⁷ In HPBLs, SeV behaves as a potent inducer of IFN- α ,⁶⁸; it induces at least nine different IFN- α species.⁶⁹ In HPBLs, SeV also stimulates the production of IFN- γ .⁷⁰ Similarly, the NDV stimulates the production of IFN- α from several gene isoforms.⁷¹ The IFN secretion can be induced by the viral Hemagglutinin-Neuraminidase (HN-protein or HN), a process independent of the double-stranded RNA (dsRNA) response, and by dsRNA replicative intermediates.^{71,72} Perhaps, the existence of two alternative parallel mechanisms of IFN induction explains the high IFN stimulating capacity of NDV^{72,73} and other paramyxoviruses.

IFNs exert complex systemic effects that make them a useful means for adjuvant therapy of cancer.^{74,75} IFNs have been used in therapeutic schemes for the treatment of metastatic melanoma, renal carcinoma, Kaposi sarcoma, bladder carcinoma, hairy-cell leukemia, etc. The significant prolongation of a disease-free survival has now been largely validated by the combined analyses of multi-institutional trials and by the subsequent studies that have included meta-analyses.⁷⁶ IFNs can act systemically by regulating the immune response through the activation of DCs, cytotoxic T cells, and NK cells. IFNs markedly increase the major histocompatibility complex (MHC) class I and class II-dependent antigen presentation that causes increased presentation of tumor-specific antigens by malignant and professional antigen-presenting cells. This presentation stimulates antitumor activity and proliferation of cytotoxic T lymphocytes.⁷⁷⁻⁷⁹

The responses to IFNs are dysfunctional in many malignancies making the direct effects of IFNs less efficient. However, IFNs can affect tumor growth indirectly, through suppression of angiogenesis

by altering the stimuli from tumor cells and by inhibiting endothelial cells. The degree of inhibition correlates with the reduced tumor vascularization and the consequent retardation of tumor growth.^{76,80,81} The treatment with IFNs may also affect the viability of cancer stem cells that are highly resistant to chemo- and radiotherapy and are responsible for the disease relapses. Most likely, IFNs affect tumor vasculature and disrupt the vascular niche of stem cells, as it was found in murine xenografts of human glioma.⁷⁵ It is suggested that IFN- γ triggered by ultraviolet (UV) inactivated SeV is responsible for lung metastasis suppression in melanoma murine model.⁸²

Stimulation of cytokines

In HPBLs, SeV was shown to stimulate the synthesis of interleukins (IL) 2, 6, 8, macrophage inflammatory protein-1 (MIP-1) α , MIP-1- β , regulated on activation normal T cell expressed and secreted (RANTES) proteins, and monocyte chemotactic protein -1 (MCP-1).^{68,70} Live or UV-irradiated SeV can stimulate the IL-6 release in treated animals.⁸³ In DCs, incubation with SeV results in the IL-6 secretion; the fusion protein (F) was found to be responsible for the effect.⁸⁴ An injection of an UV-inactivated SeV into established mouse renal cell carcinoma tumors resulted in the secretion of chemokine C-X-C motif chemokine 10 (CXCL10) by infiltrating DCs.⁸⁵ NDV was also shown to induce secretion of chemokine (C-C motif) ligand 5 (CCL5) (also known as RANTES) and CXCL10 from infected human cancer cells.³⁸ The CXCL10 was found to have an antitumor activity through the attraction of monocytes, macrophages, NK cells, T cells, and DCs. The CXCL10 also promotes T cells adhesion to endothelial cells and the inhibition of angiogenesis.⁸⁶ CCL5 and CXCL10 are T helper type 1 (Th1) chemokines and can attract activated Th1 and TCL cells into tumor nodules to exert their cytotoxicity on cancer cells.⁸⁷ In HPBLs, incubation with NDV also stimulates the secretion of cytokines such as tumor necrosis factor α (TNF- α)^{88,89} and TNF-related apoptosis-inducing ligand (TRAIL).^{71,89} It appears that the induction of TRAIL is due to a single viral HN protein.^{71,90} NDV induces the secretion of TRAIL in monocytes and NDV kills various human tumor cell lines following the stimulation of TRAIL receptors 2 (TRAIL-R2).⁹⁰ Macrophages can also be stimulated by incubation with NDV leading to upregulation of a set of macrophage-specific genes and secretion of TNF- α .⁹¹

Stimulation of NK cells

NK cells constitute a type of cytotoxic lymphocytes that bridge the innate and adaptive branches of the immune system. These cells widely interact with other components of the immune system affecting the antigen-specific T and B cell responses.⁹² The NK cells participate in the early control against virus infection and in tumor immune surveillance. These cells are responsible for a fast protective effect due to their ability to destroy abnormal or stressed cells that lack antigen-specific cell surface receptors and MHC. The NK cells do not require activation in order to kill cells, which are not expressing "self" markers of the MHC class I.

The NK cells can be activated for most efficient NK-mediated cell lysis. NK cells display natural cytotoxicity receptors that are responsible for their activation. Among these receptors are NK protein 46 (NKp46) and NK protein 44 (NKp44). Hemagglutinin neuraminidase (HN) protein of paramyxoviruses directly interacts with two receptors types: NKp44 and NKp46, and this interaction triggers the NK activation and NK-mediated cell lysis.⁹³⁻⁹⁶ In particular mumps viral HN protein mediates NKp46 activation of NK cells, and this

activation results in tumor rejection via NK-DC crosstalk.⁹⁷ Studies with the UV-inactivated SeV virus also highlight the important role of NK cells in virus-mediated oncolysis. In a mouse renal cancer model, UV-inactivated SeV produced strong anticancer effect, which was compromised by coinjection of an NK-depleting anti-mono-sialo-tetra-hexosyl-ganglioside 1 (GM1) antibody.⁸⁵

Stimulation of DCs

DCs are professional antigen-presenting cells that can efficiently stimulate innate as well as adaptive immune responses against various pathogens and cancer cells. After sensing a virus or other pathogens, the DCs enter a maturation program and become competent for education of naive T cells.

The *ex vivo* infection of DCs by recombinant SeV can induce DCs maturation and activation in only 1 hour.⁹⁸ Treatment with activated DCs harboring different variants of recombinant SeV can dramatically improve the survival of animals inoculated with cells of malignant melanoma,^{82,99} colon carcinoma,¹⁰⁰ squamous cell carcinoma,¹⁰¹ hepatocellular carcinoma, neuroblastoma, and prostatic cancer.¹⁰² The administration of such DCs before tumor inoculation has demonstrated a preventive effect against lung metastasis of neuroblastoma¹⁰³ and prostate adenocarcinoma.¹⁰⁴ Another response of DC cells to virus infection is inhibition of immunosuppression mediated by the regulatory T-cells. In experiments with the UV-inactivated SeV, carbohydrates of the viral fusion (F) protein are recognized by an unknown receptor(s) on DC cells leading to the nuclear factor kappa-light-chain-enhancer (NF- κ B) activation of B cells and secretion of IL-6.⁸⁴

Stimulation of tumor-specific cytotoxic T-cell mediated activity

Cell surface binding of NDV can stimulate the tumor-specific cytotoxic CD8+ T-cell (CTL) response and increase CD4+ T-helper cells' activity even in the absence of an antiviral T-cell response and cell infection.¹⁰⁵ The activation and infiltration of tumor sites with CTLs is the result of complex effects of virus triggered immune system, including local and systemic release of IFNs and other cytokines. Interestingly, UV-inactivated NDV was as active in promoting the tumor-specific CTL response as the replication competent NDV. It was found that this effect is mediated by the presence on the tumor cell surface of functional viral HN molecules that have affinity to plasma membranes and therefore can mediate a strong adhesion of the infected cells to CTLs.¹⁰⁵ Since the HN proteins of many paramyxoviruses are homologous, it is likely that the HN protein, regardless of whether it derives from NDV or other related paramyxoviruses, can activate both NK and CTL responses.

Viral sialidase activity of the HN protein

Metastatic cancer cells often express a high density of sialic acid-rich glycoproteins that increase the invasive potential.¹⁰⁶ The overexpression of sialic acid on the surfaces creates a negative charge on cell membranes, leading to repulsion between cells that promote metastases by helping cancer cells entry into the blood stream. Indeed, the ability of tumor cells to metastasize correlates with the abundance of sialic acids on the surface of many types of malignant cells.^{106–108} The extent of cell surface poly-sialylation was suggested as a marker characterizing the differentiation status of thyroid and small-cell lung carcinoma cells.¹⁰⁹

A more detailed analysis reveals qualitative differences in sialic acids on the surface of cells displaying different degrees of invasiveness. While in normal human colon cells, as well as in adenomas and

several carcinomas of different grades, an alpha 2, 3-linked sialic acid is detected, in the highly malignant variants, alpha 2, 6-linked sialic acids are also present. It was found that the malignant progression was associated with the *de novo* expression of an alpha 2,6 sialyl-transferase, which transforms the alpha 2,3-linked sialic acid into the 2,6-linked sialic acid.¹¹⁰ The increase in alpha 2,6 sialylation coincident with tumor progression is detected for hepatocellular¹¹¹ and colon carcinomas.¹¹²

Inhibitors of the sialylation process decrease the malignancy of cancer cells and now are considered as candidate drugs against metastatic cancer.¹¹³ One of the possible mechanisms linking the increased sialylation with malignant phenotype is the creation of a thick "coat" on the cell surface that hides cancer antigens and provides an escape of malignant cells from the immunosurveillance. For example, it is found that the tumor specific Lewis antigen of malignant medullary thyroid cancer can be masked from the immune system by both alpha 2,3- and alpha 2,6-linked sialic acid residues.¹¹⁴ Removing some sialic acid residues from the surface of malignant cells by sialidase can unmask cancer-specific antigens and make cells visible to the immune system (Figure 3). The removal of sialic acids from tumor cells is associated with a reduced growth potential, an activation of NK cells, and a secretion of IFN- γ .¹¹⁵

The HN proteins present in SeV, NDV, and some other paramyxoviruses possess both an erythrocyte agglutination and a neuraminidase (sialidase) activities.^{116,117} Neuraminidase is capable of cleaving and removing sialic acid residues from the surface of malignant cells leading to a dramatic increase in their ability to induce the T-cell response.¹¹⁸

SUMMARY OF ANTICANCER IMMUNO-TRIGGERING ABILITIES OF ONCOLYTIC PARAMYXOVIRUSES

A number of molecular mechanisms are responsible for the anti-cancer immuno-triggering abilities of oncolytic paramyxoviruses. One of them is related to viral modulation of gene expression related to antigen presentation that results in enhancement of the immunogenicity of cancer cells. Another is related to induction of autophagy in infected cancer cell that also enhances cancer cells immunogenicity. Viral sialidase activity of paramyxovirus HN protein might also contribute to improvement of cancer cell visibility for immune system by unmasking cancer antigens from a "thick coat" of sialic acid polymers. In addition, oncolytic paramyxoviruses

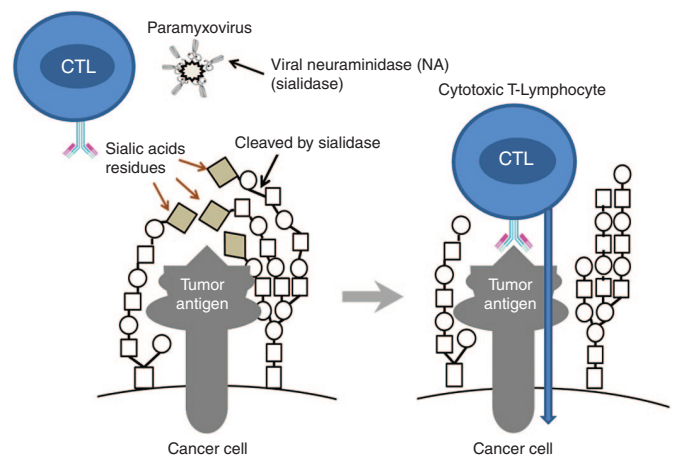


Figure 3. A hypothetical role of viral sialidase in activation of cytotoxic T-lymphocyte response against cancer cells. The removal of sialic acids may unmask hidden tumor antigens and make them more visible by the immune system.

stimulate anticancer NK cells and tumor-specific cytotoxic T cell-mediated activity. They also trigger activation of DCs, which are responsible for better cancer antigen presentation.

Strong immune activation by paramyxoviruses is a double edged sword. On one hand, it triggers strong anticancer immunity. On the other hand, it can inhibit replication of paramyxoviruses and trigger their fast clearance from a patient's body. So, perhaps a panel of different oncolytic viruses that represents different families instead of one particular virus should be used in a sequential way for maximizing a chance of complete remission during a course of oncolytic virotherapy.

COMPARISON OF ONCOLYTIC PARAMYXOVIRUSES WITH OTHER ONCOLYTIC VIRUSES

Many oncolytic viruses that belong to various viral families are currently being tested in clinical trials.^{17,119} It is becoming clear that oncolytic effects can be produced by most animal viruses having quite different genome organizations: from small positive-strand RNA-containing picornaviruses,^{120–123} small double-stranded (ds) RNA reoviruses,¹²⁴ small ds-DNA parvoviruses,¹²⁵ to medium-size ds-DNA adeno-^{126,127} and herpes viruses,¹²⁸ negative-strand RNA rhabdo-¹²⁹ and paramyxoviruses^{73,130} as well as very large poxviruses.^{131,132} The high degree of diversity among these agents suggests that while there could be common mechanisms of oncolysis characteristic to all viruses, certain specific mechanisms could be associated with particular groups of viruses.

Are there any advantages in using paramyxoviruses versus representatives of other viral families as oncolytic agents? We believe that the ability to trigger syncytium formation is a great advantage of oncolytic paramyxoviruses. As mentioned earlier (see section *Oncolysis mediated by direct cancer cell killing through formation of syncytium*), paramyxoviruses have a mechanism of spreading infection without the release of mature virus particles from cells. This includes fusion of infected cells with their noninfected neighbors. The fusion results in the formation of a syncytium from 50–100 neighboring cells (Figure 2).²⁶ The ability of the viruses to induce the formation of syncytia correlates with their oncolytic potential. Most likely, syncytia formation contributes to the efficiency of viral oncolysis because it allows extra rounds of viral replication without any exposure to host neutralizing antibodies. Moreover, malignant cells death through syncytia fusion is highly immunogenic and resembles autophagy (see section “role of autophagy”). Fused tumor cells secrete an abundance of “syncytiosomes,” which are exosome-like vesicles that highly efficiently present TAA via MHC molecules.^{27,33} In addition, cross-presentation of TAAs by DCs is strongly promoted by paramyxoviral fusogenic membrane glycoproteins.²⁷

Another potential unique advantage of some paramyxoviruses (Mumps, NDV, and SeV) is the sialidase (neuraminidase) activity of their HN protein which has the potential to remove sialic residues from the surface of tumor cells (see section *Viral sialidase activity of the HN protein*). Such removal is associated with reduced growth potential of malignant cells, activation of NK cells, and secretion of IFN- γ .¹¹⁵

FUTURE PERSPECTIVES

Improving tumor-specific targeting

NDV, SeV, and Mumps viruses can target a variety of cancers because they use sialic acids residues as their cell receptors. However, these viruses are also able to bind sialic acid residues on normal cells. So, antibodies that diminish viral binding to normal cells and increase viral binding to malignant cells could be very useful for improving

target specificity. Such bi-specific antibodies have been developed for NDV. These antibodies indeed increase NDV target tumor cells specificity in animal model experiments.¹³³

A number of studies for increasing target specificity were also performed with MVES. Even though CD46 and nectin 4 receptor binding already provides some malignant cell target specificity for MVES, it could be further increased through variable routes described below.

A series of studies on retargeting oncolytic strains of MVES employed viral attachment hemagglutinin (H) protein,¹³⁴ which is responsible for the receptor recognition and the binding specificity.¹³⁵ To kill interaction of the H-protein with CD46, the region responsible for the binding is mutagenized, and a variety of ligands that bind to selected targets of potential host cells are introduced. The ligands successfully tested in this system include the antigen-binding peptides (single-chain antibodies) against epidermal growth factor receptor (EGFR; synonyms ErbB-1; HER1 in humans),^{136–138} HER-2/neu,¹³⁹ folate receptor α ,¹⁴⁰ CD20,¹⁴¹ CD38,¹³⁷ a peptide echistatin that binds to integrins,¹⁴² and cytokine IL-13.¹⁴³

Optimization of syncytia formation and F protein cleavage site

Ability of paramyxoviruses to form syncytia increases viral oncolytic potency. The F protein of paramyxoviruses is responsible for viral fusion with the cell membrane. It is also responsible for viral spread from cell to cell via formation of syncytia. The F protein cleavage site is a determinant of virulence for different NDV viral strains.^{144,145} The introduction of the multibasic cleavage site into the F protein allows this protein to be cleaved and to be activated by a broad range of proteases. To increase oncolytic potency of one NDV strain, a polybasic cleavage site was introduced into the F protein to generate a new site. While the resultant virus exhibited only an intermediate virulence phenotype based on a mean death time in embryonated eggs, the virus formed large syncytia and was enhanced in its replication in cancer cells, leading to enhanced oncolytic effects in various animal tumor models.^{146–152}

Similar findings were shown when the F protein of the NDV La Sota strain was modified in an analogous fashion.^{144,153} The fusogenic and oncolytic activity of the rNDV/F3aa strain was further enhanced by a point mutation in the F protein at residue 289 from leucine to alanine, generating rNDV/F3aa (L289A). In an immunocompetent liver tumor rat model, administration of the mutant virus via hepatic arterial infusion resulted in improved syncytia formation and necrosis. This improvement in syncytia formation caused a significant 20% prolongation of survival.^{73,146}

Introduction of specific cleavage sites into the F protein allows utilization of tumor tissue-specific proteases, as demonstrated with SeV.^{21,154,155} Introduction of a ubiquitously recognized cleavage-motive into the F protein of SeV enables its continuous spread in malignant human tissues. A tumor-specific spreading of such recombinant SeV without evidence of bio-distribution into nonmalignant tissues⁶⁶ was found in a hepatoma xenograft animal model. Such viral constructs represent candidates for clinical trials.

Combination with autologous cell vaccines: a personalized oncolytic viral immunotherapy

“A therapeutic, autologous, antigen-presenting cell vaccine (Sipuleucel-T) was recently approved by the US Food and Drug Administration for metastatic prostate cancer, after demonstration of prolongation of overall survival in a randomized, double-blind, placebo-controlled study.”¹⁵⁶ Combination of autologous vaccine with an oncolytic paramyxovirus could provide extra benefit

for specific antitumor immune stimulation. The use of autologous tumor tissue for development of a personalized oncolytic viral immunotherapy should be further exploited with oncolytic paramyxoviruses.

Individual viral sensitivity test

There is a growing interest in personalized cancer therapy, which involves identifying those treatments which may work best for an individual's cancer. Individual viral sensitivity testing should involve testing an individual's cancer cells in the laboratory to see which virus demonstrate the best response. It therefore could provide guidance about which oncolytic virus representative may be best for the individual in clinical practice.

CONCLUSIONS

The described mechanisms of oncolysis mediated by paramyxoviruses indicate that these viruses represent exceptionally attractive instruments for cancer therapy. The genomes of paramyxoviruses are comparatively stable. The viruses induce efficient syncytium-mediated lysis of cancer cells and they elicit strong immunomodulatory effects, which dramatically enforce immune surveillance and anticancer resistance. The use of nonhuman paramyxoviruses, such as NDV, or SeV, or attenuated vaccine strains of MV, or Mumps viruses hold great advantage, as these virus strains are not associated with any serious pathology in humans.

Fast screening tests of biopsy materials are needed because variable malignancies might support a particular virus representative reproduction to a different level. Such tests should reveal which virus kills a particular malignancy in the most efficient way. In other words, an experimentally verified matching of a particular malignancy with a particular virus is highly desirable. In case several different virus representatives (perhaps even from a few different viral families) would be found to be efficient for one type of malignancy, they all might be used for virotherapy in a sequential or parallel way. Because of the potential for fast immune-mediated clearance of a particular virus from a patient, the sequential therapy may ameliorate treatment efficiency decline due to such clearance.

CONFLICT OF INTEREST

Olga V. Matveeva is a co-author of a patent entitled "Method for cancer immunotherapy and pharmaceutical compositions based on oncolytic non-pathogenic Sendai virus."

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