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The antitumor effects of Newcastle disease virus on glioma

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Abstract: Glioma is the most common primary malignant brain tumor with a poor survival rate. In recent years, no significant progress has been made in the treatment of gliomas in contrast to the development of improved diagnosis via molecular typing. Newcastle disease virus (NDV), a negative-stranded RNA virus that exhibits oncolytic activity, has been investigated for its capacity to elicit antitumor activity in many types of cancers, including glioma. Therefore, application of oncolytic viruses, such as NDV, as a new treatment strategy to specifically target aberrant signaling in glioblastomas has brought new hope. For many years, NDV has been investigated for its *in vivo* and *in vitro* efficacy in the treatment of various tumor cells. Based on its safety in humans, specificity for tumor cells, and immunostimulatory properties, NDV represents a promising antitumor agent. In this review, we summarize the background of NDV and the antitumor mechanisms of NDV-mediated oncolysis, discuss the potential value and role of NDV in gliomas, and describe new advances and perspectives for future research.

Introduction

Tumors are becoming a primary cause of death in humans, with gliomas representing the most aggressive and lethal primary brain tumor of the adult central nervous system associated with poor prognosis. Gliomas account for 24.7% of all primary brain tumors and 74.6% of malignant brain tumors (Mat and Zulkarnain, 2019). Patients with glioma are routinely treated with surgical resection combined with radiotherapy, temozolomide-based chemotherapy, and other comprehensive treatments. The prognosis after traditional treatment for glioma patients remains poor; therefore, it is urgent to identify new treatment options.

Recent advances have enabled the development of new therapeutic approaches, including oncolytic virotherapy, with the discovery of oncolytic viruses providing a novel approach to treating tumors. Moreover, oncolytic virotherapy might represent a promising form of gene therapy for the treatment of various cancer types through utilization of a combination of viral oncolytic properties and functional genes to destroy malignant cells. More than a dozen species of oncolytic viruses have been identified, including reovirus, adenovirus, herpes simplex virus, Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), autonomous microvirus, Epstein-Barr virus, measles virus, and poliovirus (Wollmann *et al*., 2012).

NDV, an oncolytic agent derived from poultry, is almost non-pathogenic to humans. People infected with NDV can cause mild conjunctivitis or lymphadenitis, which can be recovered in a short time (Bukreyev *et al*.,2005). Selective replication in human tumor cells, as well as direct tumor lysis and immune stimulation, make NDV a promising antitumor agent. NDV has been used in the treatment of glioma for > 25 years, with the first study of NDV-specific treatment of gliomas performed in 1995 (Naujocks *et al*., 1995). Current studies have confirmed the efficacy of NDV for glioma treatment. This review summarizes the background of NDV and the antitumor mechanisms associated with NDV-mediated oncolysis, discusses the potential value and role of NDV in the treatment of gliomas, and provides insight into new advances and perspectives for future research.

NDV Background

Oncolytic viruses can selectively destroy tumor cells without damaging surrounding normal tissue. Oncolytic virotherapy is a relatively new therapeutic strategy directed against various cancers, including malignant gliomas, and that shows increasing promise (Russell *et al*., 2012). Oncolytic viruses are used to recognize and infect mutated tumor cells, followed by their replication and release of new virions that directly amplify the input dose to exert antitumor effect (Niemann and Kuhnel, 2017). Additionally, newly produced virions can spread and infect nearby cancer cells. Although the use of viruses to treat tumors first started in the 1950s, it did not initially undergo significant development owing to severe toxicities associated with viral infection.

Newcastle disease virus was derived from a chicken pathogen (Beaudette, 1946), with the first reports of Newcastle disease coming from the Dutch East Indies in 1926, followed shortly by an outbreak at Newcastle on Tyne in England, from which the disease derived its current name.

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NDV exhibits selective oncolytic properties and replicates in tumor cells, where it stimulates T cell-, macrophage-, and natural killer (NK) cell-mediated responses. In 1965, Cassell and Garrett (1965) first used live NDV to infect a patient with cervical carcinoma, and oncolytic NDV has been demonstrated as a potent and safe antitumor agent for treating glioma. Defects in antiviral immunity, often present in glioblastoma (GBM), likely play a key role in determining the NDV-related tumor selectivity. Cheng *et al.* (2016) described the potential therapeutic pathways associated with oncolytic NDV tropism in GBM, providing insight into the natural selectivity of NDV toward GBM and its role in attenuating GBM proliferation and invasion signaling.

NDV is an avian paramyxovirus and a non-segmented, single-stranded, negative-sense RNA virus harboring a ~15-kb genome (Sinkovics and Horvath, 2000) with a structural arrangement of 3'-NP-P-M-F-HN-L-5' arrangement (Schirrmacher *et al.*, 2017) that encodes six main structural proteins [nucleocapsid (NP), phosphoprotein (P), large protein (L), envelope matrix protein (M) and the hemagglutinating surface glycoprotein (HN) and fusion (F)] and two nonstructural proteins (V and W). Genomic RNA is packaged by NP proteins, the L protein functions as an RNA-dependent polymerase, and the M protein aligns virions beneath the cell membrane prior to budding. Additionally, HN proteins are responsible for hemagglutinating and neuraminidase activities, and antibodies targeting HN are capable of neutralizing viral infectivity. The F and HN proteins are important envelope glycoproteins located on the NDV surface and that play important roles in viral immunity (de Leeuw and Peeters, 1999). M, F, and HN proteins are capable of forming a lipid-glycoprotein envelope on the NDV surface, with the F and HN proteins located on outer surface of the envelope subsequently mediating viral invasion and release (Wilden *et al.*, 2009). Moreover, cell fusion and syncytium formation are consequential to the interaction of the HN and F proteins (Waning *et al.*, 2002), as upon HN binding to its cell-membrane receptor, both proteins undergo conformational changes to release hydrophobic fusion peptides. Additionally, the F protein can fuse its own envelope with the host envelope to mediate the fusion of infected cells with adjacent cells, thereby making it a key protein involved in determining virulence. Furthermore, the HN protein recognizes and binds sialic acid receptors on the cell membrane through hemagglutinin, after which F and HN cooperate to promote viral entry and destroy sialic acid receptors to facilitate the release of new viral particles. Protein M, located on the inner surface of the envelope, is mainly involved in viral assembly and release (Duan *et al.*, 2017), whereas NP, P, and L located inside the NDV envelope bind RNA to form a nucleocapsid, with NP used for RNA packaging (Cheng *et al.*, 2016). Additionally, the P and L proteins constitute the RNA polymerase, which promotes viral transcription and replication, and the V proteins acts as an antagonist of interferon (IFN). There are few studies related to the roles of the W protein due to its relatively low concentration (Ravindra *et al.*, 2009).

According to NDV pathogenicity, 18 strains from four lineages can be divided into velogenic, mesogenic, and lentogenic strains (Schirrmacher *et al.*, 2009). The F protein is a major determinant of virulence (Heiden *et al.*, 2014), and introduction of a polybasic cleavage site into the F protein improves the lytic activity of lentogenic NDV strains. Introduction of a fusogenic NDV F protein into VSV significantly improved syncytia formation during NDV-mediated lysis of cancer cells (Abdullahi *et al.*, 2018). The two most widely used NDV strains (MTH-68 and HUJ) have been evaluated in clinical trials and patented for antineoplastic purposes. MTH-68/H is a live attenuated oncolytic viral strain previously used to successfully treat different tumors, including gliomas after conventional modalities had failed (Csatary *et al.*, 2004). Previous studies confirmed that MTH-68/H exerts its antitumor activity by directly and selectively attacking and killing tumor cells (Fabian *et al.*, 2017; Hrabak *et al.*, 2006). HUJ is a new lentogenic strain (NDV-HUJ) that is not pathogenic to poultry and exhibits selective cytopathogenicity for cancer cells (Yaacov *et al.*, 2008). Freeman *et al.* (2006) found that NDV-HUJ inhibits metabolic activity and promotes apoptosis in C6 and RG2 rat malignant glioma cells. Additionally, progeny NDV-HUJ particles are not infectious and can induce a cytokine-mediated immune response in addition to their direct cytotoxicity (Freeman *et al.*, 2006).

The NDV V4UPM strain is a modified V4 strain developed as a thermostable feed-pellet vaccine for poultry (Zulkifli *et al.*, 2009). Additionally, a previous study reported that the NDV D90 strain exhibits an antiproliferative effect on the human lung cancer cell line A549(Chai *et al.*, 2014). Moreover, several NDV strains induce apoptosis in many human neoplastic cells (Liao *et al.*, 2017). NDV has been confirmed as safe and effective in preclinical studies and clinical trials (Ghrici *et al.*, 2017; Bian *et al.*, 2011), and live oncolytic NDV strains have been shown to selectively replicate in tumor cells while leaving normal cells uninfected, with these strains also verified as stable, safe, and well-tolerated by patients along with minimal side effects (Reichard *et al.*, 1992). These findings in various NDV strains confirm its anticancer efficacy.

NDV-Mediated Antitumor Mechanisms

Direct mechanisms

Cancer is a primary cause of human mortality, and identification of novel treatment strategies is required to provide alternatives to the toxicity associated with radiotherapy and chemotherapy. The use of oncolytic viruses has become a hot research topic due to their selective killing of tumor cells. The first use of NDV as an antitumor agent was reported by Wheelock and Dingle (1964) for the treatment of acute leukemia. Oncolytic NDV viruses destroy host cells through direct and indirect methods (Wagner *et al.*, 2006), with NDV interacting with tumor cells resulting in their lysis, apoptosis, and initiation of an immune response.

Apoptosis

In gliomas, the antitumor IFN response is impaired by glioma-specific immunosuppressing factors such as transforming growth factor (TGF)-β, interleukin-10, prostaglandin E2, and gangliosides (Silginer *et al.*, 2017). TGF-β is the most prominent immune suppressor and plays

an important role in glioma biology while also acting as a glioma marker (Zemp *et al*., 2010). IFN-β is the principle antiviral factor secreted by infected cells; therefore, NDV replication is promoted in IFN-defective tumor cells rather than normal cells. Several studies demonstrate that NDV is selectively cytotoxic to tumor cells but not normal cells owing to tumor-specific defects in IFN antiviral responses (Abdullah *et al*., 2014). Indeed, NDV-mediated induction of apoptosis occurs independent of IFN signaling. Additionally, Wilden *et al*. (2009) reported that cell susceptibility to NDV infection is negatively correlated with basal expression of the antiviral genes *retinoic acid-inducible gene 1*, *IFN-regulatory factor* (*IRF*)*3*, *IRF7*, and *IFN-β* (Wilden *et al*., 2009). These genes are overexpressed in normal cells, which explains their resistance to NDV infection and NDV selectivity for tumor cells. Apoptosis of NDV-infected tumor cells is induced through either intrinsic or extrinsic pathways, with a previous study demonstrating that activation of the extrinsic pathway occurred earlier than the intrinsic pathway (Ravindra *et al*., 2009). However, Elankumaran *et al*. (2006) reported that the intrinsic pathway is more important to NDV-induced apoptosis, as NDV infection of tumor cells increases the production of tumor necrosis factor (TNF) α, soluble TNF-related apoptosis-inducing ligand (TRAIL), and caspase-8. On the other hand, NDV infection reportedly promotes the loss of mitochondrial membrane potential and the release of cytochrome C, followed by upregulation of caspase-9 levels, which is critical for activation of the intrinsic pathway associated with NDV-mediated apoptosis (Igney and Krammer, 2002). Moreover, pathways associated with endoplasmic reticulum (ER) stress might also be involved in the initiation of tumor-cell apoptosis (Fabian *et al*., 2007). These findings suggest that NDV-mediated apoptosis of tumor cells occurs through both the intrinsic and extrinsic pathways.

Autophagy

NDV exerts antitumor effects by inducing apoptosis during the late stages of infection (Washburn and Schirrmacher, 2002); however, the interaction between NDV and host cells is not well understood during the early stage of infection. Members of the family Paramyxoviridae induce autophagy in tumor cells, with this process associated with both the innate and adaptive immune responses to infection by microbial pathogens, including viruses. To prevent rapid elimination from tumor cells during the initial phase of infection, it is possible that the mechanisms underlying NDV-tumor-cell interaction during the early stage of infection might be related to autophagy, which is a mechanism by which damaged organelles and proteins are delivered to lysosomes, sequestered in the cytoplasm, and removed for recycling (Levy *et al*., 2017).

Bulk cytoplasm and cytoplasmic organelles are engulfed by double-membraned autophagosomes, which fuse with lysosomes to produce single-membraned autophagolysosomes to degrade the content. Autophagy plays a crucial role in both the innate and adaptive immune responses to viral infection, and Meng *et al*. (2012) reported that NDV induces an autophagic response in U251 glioma shortly after infection, and that knockdown of genes critical for autophagosome formation markedly reduces NDV production (Meng *et al*., 2012). Additionally, Takeuchi *et al*. (2005) reported that Akt signaling is involved in autophagy induction in malignant glioma cells. And that Akt phosphorylation is increased in NDV-infected cells, thereby suggesting that the class I PI3K/Akt/mTOR signaling pathway was activated during NDV infection and may not contribute to NDV-triggered autophagy (Meng *et al*., 2012). Moreover, levels of beclin-1 (BECN1) are elevated during the early stage infection, indicating that phosphoinositide 3-kinase (PI3K)/BECN1 pathway plays an important role in NDV-induced autophagy (Meng *et al*., 2012). Furthermore, suppression of autophagic molecules in NDV-infected tumor cells results in reduced viral yield, suggesting that autophagy might be utilized by NDV to promote its virulence (Meng *et al*., 2012). These findings suggest autophagy as an important direct mechanism of NDV-related antitumor activity; however, additional studies are required to determine whether NDV-induced autophagy is associated with the induction of apoptosis in NDV-infected tumor cells.

Indirect mechanisms

Beyond its direct cytopathic effects, NDV-related antitumor activity is associated with activation of both the innate and adaptive immune responses (Zamarin and Palese, 2012). As the virus replicates in tumor cells, it increases the expression of viral antigens on the tumor surface. Following infected by different NDV strains, macrophage enzymes and the production of nitric oxide and TNF-α are upregulated, suggesting that NDV infection promotes macrophage-specific antitumor activities. Additionally, NDV Ulster-stimulated monocytes mediate tumor-cell killing via TRAIL upregulation (Song *et al*., 2013; Washburn *et al*., 2003). Another study reported that IFN-β, chemokine ligand-5, IFN-γ-induced protein-10, intercellular adhesion molecule-1, and lymphocyte-function-associated antigen-3 are upregulated following NDV infection, along with increased surface expression of major histocompatibility complex (MHC), and that NK-cell activation is implicated in NDV-mediated cytotoxicity (Washburn and Schirrmacher, 2002; Zamarin and Palese, 2012; Schwaiger *et al*., 2017). Moreover, transfection of a construct expressing the HN protein induced IFN-α and TRAIL secretion, resulting in elevated levels of apoptosis in the transfected cells and suggesting that a DNA vaccine expressing the NDV HN proteins might by efficacious for antitumor therapy (Zeng *et al*., 2002). Similarly, Haas *et al*. (1998) reported that proteins HN and F expressed in NDV-infected tumor cells upregulate levels of the T cell-activation markers CD69 and CD25 specifically in tumor cells (Haas *et al*., 1998), and that these viral proteins modify the tumor-cell surface by introduction of new adhesion molecules capable of lymphocyte interactions and inducing cytokines and chemokine expression (Schirrmacher *et al*., 1999). Furthermore, Koks *et al*. (2015) reported that NDA virotherapy against GL261 tumors induces a long-term, tumor-specific immunological memory response, and that this was dependent upon the induction of immunogenic cell death (ICD). These findings suggest that NDV engineering represents a promising strategy to improve its immunostimulatory activity against tumor cells.

Further investigation of NDV-related proteins is important to identify other potential antitumor mechanisms. The antitumor effect of NDV is primarily associated with the envelope glycoprotein HN (Jarahian *et al*., 2009), which exhibits neuraminidase activity involved in antigen presentation on the tumor-cell surface to promote recognition by immune-surveillance cells. Jarahian *et al*. (2009) reported that activation of NK cells was induced by NDV-infected tumor cells presenting a ligand structure recognized by an NK cell receptor (i.e., NDV HN acts as a ligand structure for NKp44 and NKp46) (Mustafa *et al*., 2013). In line with recognition of sialic acid moieties by the HN protein, NKp44-Fc and NKp46-Fc binding is lost following desialylation. These findings suggest that NK cells stimulated by NDV-infected tumor cells produce increased amounts of lymphokines, IFN-γ, and TNF-α, suggesting HN as an important immunogen related to NDV-specific oncolytic activity associated with immune activation against tumor cells.

NDV-Mediated Antitumor Effects Against Glioma

Glioma is the most malignant tumor of the central nervous system and arises from glial cells, with grade IV (GBM) the most common primary brain tumor relative to other gliomas. Previous studies described the signaling pathways responsible for glioma initiation, migration, and invasion, thereby introducing new targets for molecular-based targeted therapy. Signaling associated with GBM proliferation emerges after mitogenic signaling, followed by the synthesis cell cycle-dependent kinases via the Raf/extracellular-signal-regulated kinase/mitogen-activated protein kinase or PI3K/Akt pathway, and subsequent aberrant cell proliferation (Abdullah *et al*., 2014). Therefore, NDV infection represents a novel biological agent capable of specifically targeting aberrant signaling in GBM.

The abnormal proliferation and aggressive invasiveness of GBM might be related to aberrant Ras-related C3 botulinum toxin substrate 1 (RAC1) protein signaling. Rac1 is monomeric G-protein Rho GTPase and a key contributor to cell survival in GBM through its regulation of cell migration and cell-cycle progression (Mustafa *et al*., 2013). Additionally, RAC1 signaling plays an important role in the initial steps of the viral life cycle (Schowalter *et al*., 2006). Puhlmann *et al*. (2010) reported that NDV is preferentially replicated in RAC1-activated cells. Moreover, the direct involvement of RAC1 in NDV-related GBM treatment involves two mechanisms: 1) endocytosis resulting in viral entry and 2) syncytium formation (Abdullah *et al*., 2014). The latter requires cell-to-cell fusion induced by NDV via HN recognition and attachment to cell-membrane receptors, followed by fusion of the F protein envelope with the host envelope and introduction of the viral nucleocapsid into the cell. NDV can also enter an infected cell through caveolae-mediated endocytosis. Caveolae are small, flask-shaped invaginations in the plasma membrane that mediate endocytosis, and RAC1 is an upstream regulator caveolae through its induction of caveolin accumulation at RAC1-positive peripheral cellular adhesion sites (Nethe *et al*., 2010). Furthermore, NDV infection induces syncytium formation as a result of cell-to-cell fusion and through the accumulation of proteins HN and F. Mansour *et al*. (2011) found that NDV-related oncolytic selectivity for tumor cells is dependent upon tumor-cell resistance to apoptosis, which fusion with neighboring cells and enhanced syncytium formation. This process prolongs the survival of cancer cells to allow the virus to replicate in the absence of an antiviral response, thereby suggesting RAC1 as essential for NDV sensitization and replication in tumor cells. In agreement with this finding, Puhlmann *et al*. (2010) reported that *RAC1* overexpression results in a significant increase in NDV replication accompanied by increased oncolysis. Interestingly, another study described time-dependent upregulation of RAC1 levels at 3-, 6-, and 9-h post-infection with NDV, followed by a significant downregulation at 12-h post-infection (Mustafa *et al*., 2013). In glioma cells, depletion of RAC1 strongly inhibits lamellipodia formation and leads to a decrease in cell migration and invasion (Nakada *et al*., 2011). Additionally, this study showed that inhibited RAC1 activity induces apoptosis in primary and glioma cell lines but not in normal adult astrocytes. These findings show that RAC1 promotes NDV entry, syncytium induction, and replication (Abdullah *et al*., 2014).

Ali *et al*. (2011) reported NDV strain AF2240 as a potent antitumor agent that induces apoptosis in an anaplastic astrocytoma brain tumor cell line, and that cytotoxicity increased according to viral titer. Additionally, Zulkifli *et al*. (2009) revealed that NDV strain V4UPM inhibits the proliferation of glioma cells and promoted tumor regression and apoptosis in gliomas. Furthermore, a previous study reported that MTH-68/H selectively kills tumor cells by inducing ER stress, resulting in p53-independent apoptotic cell death (Fabian *et al*., 2007).

The two primary methods for the administration of oncolytic viruses in clinical settings for the treatment of gliomas are intratumoral and intravenous injections (Kazimirsky *et al*., 2016), while the latter one is widely used in the clinical application of NDV (Tab. 1). Csatary and Bakacs (1999) observed tumor regression and improved neurological function in a 14-year-old boy with high-grade glioblastoma following daily treatment with intravenous MTH-68/H (Csatary and Bakacs, 1999). In 2004, four patients with advanced high-grade glioma were treated with MTH-68/H intravenously, resulting in tumor regression and long-term survival (5-9 years) with good quality of life (Csatary *et al*., 2004). However, in 2006, combination therapy involving MTH-68/H and valproic acid used to treat a 12-year-old boy with anaplastic astrocytoma resulted in glioma recurrence following a 4-month regression, and continuous injection of MTH-68/H exhibited few effects on tumor regression (Wagner *et al*., 2006). Recently, a phase I/II trial using strain NDV-HUJ in 14 patients with apparent recurrent GBM revealed that intravenous NDV-HUJ was well-tolerated and displayed no significant toxicity without having reached the maximum tolerated dose (Freeman *et al*., 2006). Therefore, findings of good tolerability and encouraging responses warrant the continued evaluation of NDV-HUJ for the treatment of GBM, as well as other cancers. Furthermore, post-oncolytic antitumor immunity based on ICD is an important aspect of tumor-cell death, suggesting that the most promising and feasible method for clinical practice appears to be combined administration of systemic NDV pretreatment with antitumor

vaccination (Schirrmacher, 2015). Several studies demonstrate the therapeutic efficacy and safety of autologous NDV-modified cellular vaccines or oncolysates in clinical trials; however, most of these were small or uncontrolled studies, suggesting that larger controlled clinical trials are necessary to confirm efficacy.

TABLE 1

Summary of clinical trials with NDV for treatment of glioma

Viral strain	Route	Type of study and patient number (n)	Clinical outcome	Reference
NDV MTH-68	IV	Case report, n = 1	CR: at a high dose of MTH-68/H (8 × 10^7 PFU)	Csatary and Bakacs, 1999
NDV Ulster	vaccination	Phase I/II, n = 11	The median survival was 46 weeks (mean 60 weeks), long-term benefit in one patient	Schneider et al., 2001
NDV Ulster	vaccination	Phase II, n = 23	Improved PFS (40 weeks) and OS (100 weeks), long-term benefit in one patient	Steiner et al., 2004
NDV MTH-68	IV	Case series, n = 14	Seven patients benefit from NDV treatment, long-term benefit in 4 patients	Csatary et al., 2004
NDV MTH-68	IV	Case report, n = 1	PD: 4 months after NDV treatment	Wagner et al., 2006
NDV HUJ	IV	Phase I/II, n = 14	One patient achieves CR	Freeman et al., 2006

IV: intravenous,

CR: complete response,

PFS: progression-free survival,

OS: overall survival,

PD: progressive disease.

New Advances

Recombinant NDV (rNDV)

Oncolytic virotherapy represents a promising method for cancer treatment through utilization of viral oncolytic properties and genes to destroy tumor cells (Fan *et al*., 2018). A phase I clinical trial of the NDV lysolytic strains MTH-68/H and NDV-HUJ in gliomas has been completed (Pol *et al*., 2016); however, the use of NDV alone is insufficient to achieve a complete antitumor effect. The tumor-suppressor protein p53 plays a primary role in a number of pathways, and glioma-specific tumorigenesis is associated with genetic mutations in p53 (Ohgaki *et al*., 1993, Rivlin *et al*., 2011); therefore, dysregulation of p53 might represent a therapeutic target. A recent study reported that a p53 oncolytic agent delivered using rNDV (rNDV-p53) might improve the prognosis in mice with glioma by promoting tumor-cell apoptosis and the effects of immunotherapy, respectively (Fan *et al*., 2018). Indeed, infection with the rNDV-p53 virus markedly enhanced the transcription of p53, p21, and caspase-3 while inhibiting the expression of anti-apoptotic proteins. Additionally, rNDVp53 administration significantly induced an immune response via cytotoxic T lymphocytes and lymphocyte infiltration while increasing cancer-cell apoptosis, suggesting its potential oncolytic efficacy against glioma cells. This result indicates that combination therapy involving rNDV represents a promising therapeutic strategy for glioma.

Cell carriers

NDV is a safe antineoplastic agent according to its efficient and selective replication in and destruction of tumor cells associated with the reduced tumor-specific secretion of IFN relative that induced in normal cells (Krishnamurthy *et al*., 2006). Additionally, NDV-related oncolytic activity is associated with the intrinsic cell-death pathway and the secretion of specific cytokines. However, when oncolytic viruses delivered directly into the circulation, the viruses will face many hazards that impede their localization to, and infection of, metastatic tumors (Willmon *et al*., 2009). To improve viral delivery, a recent study demonstrated mesenchymal stem cells (MSCs) as efficient virus-delivery methods (Kazimirsky *et al*., 2016, Wollmann *et al*., 2012). Moreover, MSCs can enhance the NDV-specific oncolytic effect in glioma cells and glioma stem cells by promoting the secretion of TRAIL, which is associated with cell death in glioma cells. These findings suggest that loading of the

NDV into MSCs can promote tumor-cell targeting and enhance NDV-related antitumor effects via immunogenic activity (Kazimirsky *et al*., 2016). Targeted delivery of the virus to tumor cells plays an important role in this method while the presence of the infected cells might also enhance the local immune response (Park *et al*., 2009). Previous studies indicate the cell-carrier-mediated targeted therapy for oncolytic viruses involves three stages (Willmon *et al*., 2009): (1) loading of the viruses into cell carriers; (2) transport of the viruses to the tumor site via blood circulation; and (3) release of the viruses from the carriers cells upon reaching the tumor site. Current findings suggest this method as a promising technique for NDV delivery to tumor sites.

Vaccination

Direct infection of tumor cells with viruses transferring protective or therapeutic genes is a frequently used procedure to produce tumor vaccines in human gene therapy; however, this approach is often limited by the number of tumor cells that can reliably be infected, as well as issues of selectivity and safety (Schirrmacher *et al*., 1999). Currently, NDV offers a selective and safe method for infecting human tumor cells with a natural virus. In addition to the direct oncolytic use of NDV, its role as a human autologous tumor vaccine is emerging. Tumor vaccines are obtained from tumor cells treated with radiation and infected with the virus that can be subsequently preserved in liquid nitrogen. Currently, autologous tumor vaccines exert their therapeutic effects by activating the patient immune system via 1) inducing the production of tumor-suppressive factors, 2) activating atopic immune cells, and/or 3) nonspecific cytotoxic effects. Lentogenic NDV infection of tumor cells induces apoptosis, with the Ulster strain of NDV demonstrated as efficacious for GBM treatment (Freeman *et al*., 2006). The Ulster genome cannot be integrated into the host gene and only selectively replicates in tumor cells while also exhibiting multidirectional immunomodulatory ability (Schild *et al*., 1989). Steiner *et al*. (2004) reported that virus-modified autologous tumor cells used as a vaccine appeared both feasible and safe according to their ability to elicit an antitumor immune response, suggesting their potential ability to improve the prognosis of GBM patients (Steiner *et al*., 2004). Given that NDV elicits fewer adverse effects on humans relative to other viruses, including herpes simplex virus (type I) and adenovirus, this suggests that NDV might be efficacious for use in a vaccine for cancer therapy.

Combination therapy

Temozolomide (TMZ) is a first-line clinical chemotherapeutic drug used for the treatment of GBM; however, rapid recurrence and multidrug resistance represent major challenges associated with this drug. TMZ resistance remains a challenge associated with its use specifically in treating gliomas; therefore, more effective therapeutic strategies need to be identified, suggesting that TMZ combined with NDV might represent a possible effective method (Shi *et al*., 2016). Previous studies indicate that TMZ combined with NDV has an opposite effect on Akt signaling, with TMZ treatment capable of stimulating endogenous AKT kinase activity, and NDV infection promoting apoptosis by inhibiting AKT-related signaling (Hirose *et al*., 2005, Meng *et al*., 2014). Moreover, both NDV and TMZ activate 5'AMP-activated protein kinase associated with maintenance of cellular energy homeostasis (Bi *et al*., 2018), and a recent study indicates that combination therapy with NDV and TMZ significantly extends the survival of GBM cells by inhibiting their growth and enhancing pro-apoptotic effects while also alleviating TMZ resistance (Bai *et al*., 2018), which has great clinical significance. Moreover, Alkassar *et al*. (2011) reported that combined treatment with two viruses (reovirus and NDV) significantly enhanced oncolysis in established glioblastoma cell lines by promoting apoptosis. These findings suggest that combination therapy involving NDV might represent an efficacious option for treating patients with GBM.

Conclusion

The current standard treatment for glioma is surgical resection, followed by radiotherapy plus auxiliary TMZ. Unfortunately, the prognosis for glioma patients, especially those with GBM, is relatively poor, with a median progression-free survival of < 7 months and a median overall survival of only 15 months. Therefore, new treatment strategies are critically needed. Targeted therapy uses a specific molecule to hinder or reboot aberrant signaling in tumor cells, and application of viruses as novel biological agents to specifically target such pathways represents an effective therapeutic option. NDV is an intensively studied oncolytic virus capable of selective targeting of tumors to elicit oncolytic effects. NDV infects tumor cells through direct and indirect mechanisms that either activate extrinsic and intrinsic apoptotic pathways or autophagy processes (direct) or an immune response via activation of macrophages and NK cells, cytokine secretions, and presentation of immunospecific ligands on the tumor-cell surface (Zamarin and Palese, 2012). Additionally, NDV-related virotherapy against glioma involves attenuation of the abnormal proliferation and aggressive invasion behavior of GBM related to aberrant RAC1 signaling. However, efficient oncolytic virus delivery into the brain still presents a major problem (Pulkkanen and Yla-Herttuala, 2005), thereby how to enhance the anti-tumor immunity of NDV and improve the tumor-specific targeting of NDV is an urgent problem that needs to be solved before NDV is used in clinical treatment of glioma. Recent studies have provided critical insight into NDV-specific mechanisms associated with inhibited tumor proliferation in gliomas, and new advances, including the use of rNDV, optimized delivery method to tumor sites, vaccination, and combination therapy, have led to a progress in the use of viral therapy (Fan *et al*., 2018). Furthermore, the absence of major side effects associated with NDV infection promotes its clinical application. Further investigation into the use of NDV as a cancer therapeutic is necessary, especially glioma patients; however, recent advances in genetically engineered NDV have been identified in association with improved clinical efficacy. These findings suggest that NDV represents a novel and efficacious immunotherapeutic approach to treating gliomas owing to its safety, specificity, and immunostimulatory properties.

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