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Review Article

Interleukin-12-expressing oncolytic virus: A promising strategy for cancer immunotherapy

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الملخص

الفيروسات القاتلة للأورام السرطانية؛ هي من أنواع العلاجات المبتكرة المضادة للسرطان، التي تستهدف وتدمر الأنسجة السرطانية بانتقائية دون المساس بالخلايا السليمة. اكتسب علاج الأورام بالفيروسات القاتلة المزيد من الاهتمام للمزيد من التطور كنوع جديد من العلاج المناعي بعد موافقة إدارة الغذاء والدواء الأمريكية مؤخرا على فيروس الهربس لعلاج الورم الملانيني المتقدم. ويقوم النهج العملي لتحقيق أقصى قدر من الفاعلية للفيروسات القاتلة للأورام السرطانية على تسليح الفيروسات بالسايتوكاينز المعززة للمناعة، لتكون قادرة على تعزيز القدرة المناعية للهجوم على الخلايا السرطانية بفاعلية. وقد تم تصنيف انترليوكن -١٢ كاسايتوكاينز قوي له أنشطة مضادة للأورام فاعلة، تقوم بتنشيط كلا من الجهاز المناعي الفِطري والتَكَيُفي المضاد للأورام. وأظهرت العديد من الدراسات أن انترليوكن -١٢ ذي خصائص الفيروسات القاتلة للأورام السرطانية، يحسن مؤشر العلاج لعينات الورم قبل السريرية بواسطة تنشيط وتوظيف الخلايا الجذعية، والخلايا السامة الطبيعية القاتلة والخلايا "ت" السامة للخلايا التي بدورها تحسن إزالة الورم. في هذا العرض، نناقش آلية المناعة لعمل الفيروسات القاتلة التي تحوي انترليوكن -١٢.

الكلمات المفتاحية: العلاج المناعي؛ انترليوكن -١٢؛ العلاج بالفيروسات؛ السرطان؛ الفيروس القاتل للورم السرطاني

Abstract

Oncolytic viruses (OVs) are an emerging class of novel anti-cancer therapeutic agents that selectively infect and destroy cancerous tissues without damaging normal cells. With the recent US Food and Drug Administration (FDA) approval of Herpes Virus (T-VEC) for the treatment of advanced melanoma, oncolytic virotherapy has gained more attention for further development as a novel form of immunotherapy. A viable approach to maximize the efficacy of OVs involves arming them with immune-enhancing cytokines that are capable of boosting the host's immune response to effectively attack tumour cells. Interleukin-12 (IL-12) is a powerful cytokine with potent antitumour activities that activates both innate and adaptive anti-tumour responses. Several studies have demonstrated that IL-12-expressing OVs improve the therapeutic index in pre-clinical tumour models by activating and recruiting dendritic cells (DCs), cytotoxic natural killer (NK) cells and cytotoxic T cells, which subsequently improve tumour clearance. In this review, the immunological mechanisms of IL-12–expressing viruses are discussed.

Keywords: Cancer; Immunotherapy; Interleukin-12; Oncolytic virus; Virotherapy

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Introduction

One of the most potent alternative cancer treatments is gene therapy using various agents, such as oncolytic viruses (OVs), which are viruses that can selectively infect, replicate, and generate a greater immune response against cancer and directly kill tumour cells.[1] OVs have been used in clinical trials for the treatment of various cancers, such as pancreatic cancer, ovarian cancer, colorectal cancer and glioma.[2] OVs are designed to target and hijack cancerous cell machinery including: (a) pro-apoptotic targeting where the viruses delay apoptosis of infected cells to promote their replication for the synthesis and assembly of a large number of new viruses before killing the infected cancer cell; (b) transcriptional targeting where an essential viral gene is placed under the regulation of tumour specific promoters; (c) transductional targeting where the ability of the tumour cells to up regulate their tumour-specific receptors is exploited for specific targeting of cancer cells by OVs, and (d) targeting strategies based on the tumour microenvironment where modified microenvironments, such as angiogenesis, hypoxia and activation of certain proteases are exploited.[3] The infected cancer cells eventually produce more infectious particles that infect neighbouring cancer cells; thus, the anti-cancer "infection" spreads.

Direct tumour cytotoxicity appears to be only one of the key mechanisms mediating the anti- efficacy of OV.[4] Interestingly, immune-mediated tumour suppression for overall OV efficacy is likely influenced by the initial period of vigorous OV replication and lytic activity, which optimally set the stage for subsequent antitumour immune response.[4,5] Studies have shown that T and NK cell recruitment into tumours associated with increased survival and enhance antitumour efficacy, subsequently, genetically modified viruses coding for immunomodulatory agents, such as cytokines or chemokines, have come into focus. Such engineered viruses can promote an efficient anti-tumour immune response using several mechanisms, including the induction of intrinsic cellular stress pathways that activate innate immunity, expression of stress-induced self-ligands rendering this cell susceptible to natural killer (NK) cell-mediated lysis and the enhanced presentation of tumour-specific antigens to cytotoxic $CD8^+$ T cells.[6] A milestone has been achieved in the usage of cytokine-armed oncolytic viruses when the FDA recently approved talimogene laherparepvec (T-VEC), a herpes simplex virus (HSV) expressing the immunostimulatory cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF), in the treatment of patients with metastatic melanoma.[7] A number of OVs that harbour IL-12 have been demonstrated, in which IL-12 significantly enhances the anticancer immune response. Interleukin-12 (IL-12) is a heterodimeric cytokine produced primarily by phagocytic cells and antigen-presenting cells in response to bacteria, bacterial products, and intracellular parasites. The major target cells of IL-12 action include NK and T cells, which induce IFN-gamma (IFN-γ) production, cytotoxic activity, increased proliferation in cooperation with other costimulatory signals, and differentiation of T helper type 1 (Th1) cells.[6] IL-12 is one of the most potent anti-cancer cytokines. Its antitumoural effect is mediated by the activity of T, NK, and NKT cells and by an anti-angiogenic effect. Given its ability to activate both NK and cytotoxic T cells, IL-12 represents the ideal candidate for tumour immunotherapy in humans.

Unfortunately, systemic administration of IL-12 to cancer patients causes excessive clinical toxicity and severe side effects.[8] To avoid such severe side effects, scientists have used novel methods to deliver this cytokine directly to the tumour site, and one of these methods involves the use of OVs. Taking advantage of OV oncotropism, OVs appear to be a promising platform to effectively deliver IL-12 and restrict it is expression within the tumour microenvironment. The present review summarizes the most promising IL-12-expressing OVs as shown in preclinical models and patients.

Adenovirus

Adenoviruses belong to the family of *Adenoviridae*. Their name is derived from the human adenoid tissues from which they were first isolated.[9] Adenoviruses are non-enveloped viruses with a genome of double stranded linear DNA 26–48 kb in size and a capsid of 65–80 nm in diameter.[9] The capsid contains penton, hexon and fibre proteins that are important for the infection of cells by the virus. The virus uses receptor-mediated endocytosis to enter cells.[10] The first vector systems developed for gene delivery and expression were derived from adenoviruses.[11] Subsequently, adenoviruses have emerged as one of the most frequently used viral vectors for gene therapies, including cancer viral therapy.[11] This emergence is related to their ability to affect the metabolic activity of various cells (replicating and non-replicating cells), host large inserted genes, and code for proteins without incorporating into the cell genome of the host.[11]

Various adenovirus mutants, which are capable of killing tumour cells, have been engineered to utilize tumour-associated promoters to regulate the expression of essential viral genes, thus restricting viral replication and spreading to tumour cells.[12] A common strategy in designing oncolytic adenoviruses is to genetically engineer the adenoviral early region 1A (E1A).[13] This process involves a bondage of the CR2 region of adenoviral E1A to the retinoblastoma protein (RB) and other related proteins that regulate the E2F family of transcription factors, which plays a pivotal role in the regulation of cellular proliferation and stimulates the entrance of quiescent cells into the S phase.[13] Given that cancerous cells often exhibit a dysfunctional RB and uncontrolled cell cycle, deletion of the CR2 region allows the mutant adenovirus to replicate selectively in tumour cells.[14,15]

Several adenovirus mutants have been generated to maximize virus selectivity and efficacy, including mutant dl1520 (ONYX-015/CI-1042), mutant dl 922–947 and mutant AdA-24. Mutant dl1520 (ONYX-015/CI-1042) was the first engineered replication-selective virus used in humans and has been demonstrated to be an anti-cancer agent in preclinical studies and clinical trials.[16] Various studies have shown that arming oncolytic adenovirus with immune modulatory cytokines, such as IL-2 and IL-12, induce enhanced antitumour activity. Expression of the genes associated with the pre-cited interleukins induces the production of cytokines that activate the type-1 immune response, thus resulting in the regression of the development

of malignant tumours.[17] Huang et al. demonstrated that the co-expression of IL-12 and 4-1BBL via an oncolytic adenovirus significantly enhanced the antitumour and anti-metastatic effects compared with the expression of the individual genes coding for either cytokine in a preclinical study.[18] Moreover, their study revealed a synergistic enhancement in interferon (IFN)-γ levels in mice treated with adenovirus simultaneously expressing IL-12 and 4-1BBL. The latter study also reported that the increased antitumour immune response is potentially mediated via the enhanced cytolytic activity of cytotoxic T lymphocytes (CTLs) and IFN-γ-releasing immune cells. Another study investigated the antitumoural activity of a recombinant adenovirus expressing murine IL-12 (AdmIL-12) in a murine model of prostate cancer and revealed that the tumour growth was reduced by greater than 50%, whereas the survival time increased from 23.4 to 28.9 days.[19]

Herpes simplex virus (HSV)

HSV is an enveloped double-stranded DNA virus belonging to the family of *Herpesviridae*.[20] The virus infects human cells by fusion of the viral envelope proteins with the host cell plasma membrane. There are two types of HSV: HSV1 and HSV2. In the case of HSV-1, three major gene regions, alpha, beta, and gamma, are present in the genome and act simultaneously to regulate the entry, replication and spreading of the virus in host cells.[3]

To allow a replication of HSV in malignant tumour cells specifically, a variety of mutants, such as R3616 and G207, have been designed by inactivating the function of the viral genes that encode for ribonucleotide reductase, thymidine kinase and infected cell protein 34.5 (ICP34.5).[21–23] These mutants can replicate preferentially in proliferating cancer cells that exhibit a high endogenous ribonucleotide reductase activity. Phase I clinical trials using the latter mutants have revealed an effective therapy in the treatment of malignant brain tumours.[21]

The advantage of using oncolytic HSVs as agents for localized IL-12 delivery, expression, and immunomodulatory activity relies on the fact that they are tumour specific and trigger a limited antitumour adaptive immune response.[24] Tooba et al. reported the efficiency of G47Δ-mIL12, a genetically engineered IL-12-expressing oncolytic HSV, in the treatment of glioblastoma, an aggressive and fatal adult brain tumour. Using a murine glioblastoma stem cell (GSC) model they demonstrated that G47Δ-mIL12 infects and replicates similar to its unarmed oncolytic HSV counterpart *in vitro*, whereas G47Δ-mIL12 significantly enhances survival of mice with intracerebral 005 tumours *in vivo*. The study also revealed that in addition to targeting GSCs, G47Δ-mIL12 increases IFN-γ release, hinders angiogenesis by inducing IP-10 protein, and decreases the number of regulatory T cells in the tumour. G47 armed with immunomodulatory molecules, such as IL-12, significantly increases the efficacy of oncolytic HSV-1 therapy in glioma treatment.[24]

Newcastle disease virus (NDV)

NDV, also known as avian paramyxovirus-1 (APMV-1) belongs to the family of *Paramyxoviridae*. The genome is a single-stranded negative-sense RNA that is15 kb long and contains six genes encoding at least eight proteins, including six structural (NP, P, M, F, HN, L) and two non-structural (V and W) proteins.[25] NDV accesses the host by first binding to the cells via the HN protein and acid-containing host cell receptors. Further fusion of the viral and cell-surface membranes occurs followed by a release and replication of the viral RNA into the cytoplasm.[26]

NDV possesses various properties that make it an excellent anti-tumour agent, including good cell binding, entrance into the cell cytoplasm via receptor-mediated endocytosis, replication in tumour cell cytoplasm selectively and independently of cell proliferation, proven safety with no serious side effects, and possibility of used as an adjuvant.[27–29]

The oncolytic properties of NDV have been studied both in animal models and humans. Partial to complete regression of tumours was observed in both cases, even in patients with advanced tumours who were unresponsive to standard therapy.[30] The occurrence of acute or chronic side effects was negligible.[30,31] In patients suffering from breast or ovarian cancer, an enhanced survival rate was also noticed.[32]

Various studies have reported the oncolytic activity of IL-12-expressing NDVs.[33–37] Ren and his colleagues investigated and compared the antitumoural effect of genetically engineered NDV strains expressing both IL-12 and/or IL-2 (rClone30—interleukin-2, rClone30—interleukin-12, and rClone30—interleukin-12—interleukin-2).[38] Their study revealed that rClone30—interleukin-12—interleukin-2 was more efficiently inhibited hepatoma in mice compared with either rClone30—interleukin-12 or rClone30—interleukin-2 alone. Another study investigated the immunomodulatory activity of low titres of NDV AF2240 on human peripheral blood mononuclear cells (PBMC).[38]

Semliki forest virus (SFV)

SFV is an alphavirus belonging to the family of *Togaviridae*. SFV contains a single positive-strand RNA genome that replicates in the cytoplasm of infected cells.[39] The genome encodes only nine functional proteins, including four non-structural proteins that are involved in viral RNA synthesis, four structural proteins that form the capsid (the C protein) and the envelope (the E1, E2 and E3 proteins) and a small 6-kDa protein encoded by the structural region of the genome that is not incorporated into virions.[40,41] As with many other viruses, the pathogenic characteristics of SFV have been manipulated to treat diseases rather than cause them.[41] Interestingly, SFV is considered an efficient vector system to deliver and express transgenes due to its ability to replicate and grow to a high titre in cultured cells, such as baby hamster kidney (BHK) cells and chick embryo cells.[41]

Various studies have been performed to screen the oncolytic properties of SFV.[42–44] VA7-SFV, which encodes the enhanced green fluorescent protein gene (EGFP), is novel virotherapy candidate against unresectable osteosarcoma, an aggressive malignant tumour primarily noted in children and young adults that involves a proliferation of malignant mesenchymal cells producing immature bone or osteoid.[42] Their study demonstrated a profound regression in tumour size in mice treated with the vector.[42] Using a poorly

immunogenic MC38 colon adenocarcinoma model to evaluate the therapeutic potential of SFV vectors, Rodriguez-Madoz et al. demonstrated that a single intratumoural injection of two IL-12-expressing SFV vectors (SFV-IL-12 or SFV-enh-IL-12) induced greater than 80% complete tumour regression with long-term tumour-free survival.[45] However, lower doses of SFV-enhIL-12 were more effective than SFV-IL-12 in inducing antitumoural responses, demonstrating a positive correlation between the IL-12 expression level and the therapeutic effect.[45] Moreover, repeated intratumoural injections of suboptimal doses of SFV-enhIL-12 increased the antitumoural response.[45] Quetglas et al. also reported that SFV-IL-12 is effective in the treatment of liver cancer in a murine study, but the efficiency is dependent on a long-term immune response.[46]

Vesicular stomatitis virus (VSV)

VSVs are members of the family *Rhabdoviridae* and the prototypes of the *Vesiculovirus* genus. VSVs are bullet shaped with a genomic structure involving a single strand negative-sense RNA composed of five genes (N, P, M, G, and L) representing the nucleocapsid protein, phosphoprotein, matrix protein, glycoprotein, and the large protein, which is a component of the viral RNA polymerase, respectively.[47] The oncolytic activity of VSV toward various cancers, such as glioma, hepatocellular carcinoma, breast carcinoma, melanoma and lung cancer, has been demonstrated in several preclinical tumour models.[48–50] The oncolytic strategy of VSV is primarily based on a defect in the type I IFN response of numerous malignant tissues.[51,52] A disadvantage of the clinical application of VSV is associated with the potential for neurotoxicity.[53,54] This deleterious effect has been observed in many preclinical studies. However, to overcome this issue, strategies involving IL-12 combinational therapy are beneficial from a safety prospective. Derek et al., reported that IL-12 is essential to recover from VSV infection of the murine central nervous system (CNS).[55] This antiviral effect is mainly mediated by the induction of the neuronal isoform of nitric oxide synthase (NOS-1) and is independent of the proinflammatory cytokines IFN-γ and TNF-α.[55]

Shin et al. demonstrated that recombinant VSV vectors incorporating genes coding for viral fusion protein (rVSV-F) and interleukin 12 (rVSV-IL12) have significant antitumour effects against squamous cell carcinoma (SCC) in an orthotopic murine model.[51] Cultured human and murine SCC cells are good platforms for efficient replication of rVSV-F, whereas normal human and mouse keratinocytes were not suitable for the vector.[51] Injection of multiple doses of the vectors in a single SCC nodule in mice revealed that recombinants significantly contribute to the regression of the tumour and a prolongation of the animal survival.[51,52] Using an orthotopic murine SCC model, Shung et al. assessed the antitumoural activity of a combination of rVSV-F or rVSV-IL12 with systemic cisplatin (a chemotherapy agent), The combination of cisplatin with either VSV type has a positive effect on the elimination of tumour cells *in vitro*.[56] *In vivo*, the addition of cisplatin increased the antitumoural effect of rVSV-F but hindered the activity of rVSV-IL12.[56]

Sindbis virus (SINV)

Similar to SFV, SINV is a virus that belongs to the *Togaviridae* family.[57] SINV is an alphavirus with a genome composed of a positive single-stranded RNA of approximately 12 kb that encodes four non-structural and two envelope proteins.[58] SINV is transmitted by mosquitoes and is not typically responsible for severe disease in humans.[59] Thus, SINV serves as a potential platform for investigations of cancer treatment through gene therapy due to the absence of pre-existing neutralizing antibodies. Another factor that makes SINV a suitable oncolytic agent is related to the fact that its lifecycle does not involve a DNA phase, thereby eliminating any risk of genomic integration.[60] The mechanism by which SINV targets tumour cells is thought to involve interactions with the high-affinity laminin receptor, a receptor for SINV in mammalian cells.[61] This receptor is overexpressed in many cancers and makes tumour cells easy targets for the virus.[61] Another advantage of using SINV in the treatment of cancer is associated with the fact that the virus is a blood-borne pathogen and can be administered steadily to target metastatic tumours.[60]

The oncolytic properties of SINV have been demonstrated using various cells line and animal models. Using BHK tumours in SCID/beige mice, it was demonstrated that injections of a SinRep/LacZ vector decreased the size of the tumour significantly in mice.[60] Moreover, the same study revealed that the efficiency of the vector in destroying tumour cells was enhanced in the presence of NK cells. In addition, a therapeutic effect of administering IL-12-expressing SINV vectors has been demonstrated for the treatment of human ovarian tumours in SCID mice.[60] In addition, Sin/IL12, an IL-12-carrying SINV vector, has a beneficial effect on the regression of malignant tumours in mice. This vector results in an increase in the survival time, which is primarily due to IFN-γ-induced NK cell recruitment.[60]

Conclusion

A revolution in molecular and cancer biology has occurred in recent years that has allowed a better understanding of the interactions between viruses and host cells. This new understanding has also provided an impetus for the development of cancer treatment by gene therapy that involves the delivery of specific genes by OVs to efficiently target tumour cells. Numerous viruses have been investigated for their anti-cancer properties and appear to be promising in different phases of clinical trials in humans. Unlike the systemic administration of recombinant IL-12, IL-12-expressing OVs exhibited no IL-12-associated organ toxicity. Extensive animal studies on the use of IL-12-expressing OVs for cancer treatment have clearly demonstrated a significant antitumour effect of such viruses that led to partial or complete regression of cancer concomitant with an increased survival rate in murine tumour models. These studies have suggested a model of action wherein IL-12-expressing OV has multimodal effects: (a) directly on immune cells, such as cytotoxic T cells, NK cells, and DCs, and (b) indirectly through IP-10 chemokine induction, which robustly interferes with tumour angiogenesis (Figure 1). These studies have clearly demonstrated multiple mechanisms by which IL-12-expressing OVs mediates

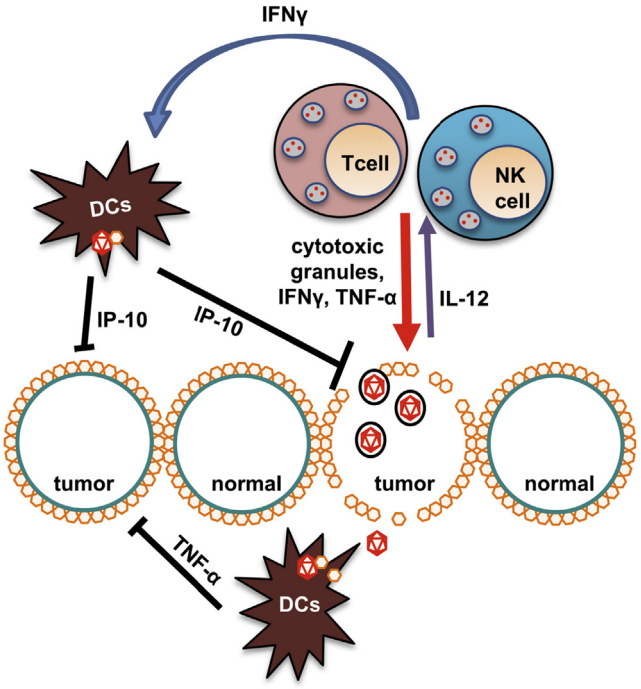

Figure 1: Interleukin-12-expressing OVs (OV-IL12) exert multimodal antitumoural activity. OVIL12 infects and spreads within tumour cells, selectively lysing tumour cells and sparing normal cells. During the viral replication, OV-IL12 releases IL-12 protein, leading to a direct and indirect cascade of events that modulate the tumour microenvironment. Direct induction typically occurs in all immune cells that express IL-12 receptors are noted on the cell surface of a variety of cells, including NK cells, cytotoxic T cells (mainly CD8 T cells) and DCs. These three subsets play major roles in either tumour cell lysis (NK and T cells) or antigen processing and presentation (DCs), which further supports the development of long-term T cell memory. An indirect impact on the tumour milieu is mainly regulated by IFNγ-induced mechanisms that act via several methods on both host and tumour cells to enhance tumour regression. Effects on the host include the upregulation of IP-10 chemokine that has a robust antiangiogenic effect on tumour cells. All together, these multifaceted antineoplastic advantages of OV-IL12 viruses induce tumour regression and ultimately tumour eradication.

tumour protection in several pre-clinical models. Therefore, an ideal IL-12-expressing OV candidate should have the potential to utilize all of the mechanisms that have been illustrated. Therefore, the research focus should be geared toward the investigation of more combinative therapies, including immunomodulatory agents, chemotherapy and radiotherapy, to achieve the optimal benefit.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

AAA and ABM contributed equally to this review.

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