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REVIEW

Oncolytic Viruses in the Treatment of Cancer: A Review of Current Strategies

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Abstract Oncolytic viruses are live, replication-competent viruses that replicate selectively in tumor cells leading to the destruction of the tumor cells. Tumor-selective replicating viruses offer appealing advantages over conventional cancer therapy and are promising a new approach for the treatment of human cancer. The development of virotherapeutics is based on several strategies. Virotherapy is not a new concept, but recent technical advances in the genetic modification of oncolytic viruses have improved their tumor specificity, leading to the development of new weapons for the war against cancer. Clinical trials with oncolytic viruses demonstrate the safety and feasibility of an effective virotherapeutic approach. Strategies to overcome potential obstacles and challenges to virotherapy are currently being explored. Systemic administrations of oncolytic viruses will successfully extend novel treatment against a range of tumors. Combination therapy has shown some encouraging antitumor responses by eliciting strong immunity against established cancer.

Keywords Oncolytic viruses · Tumor cells · Human cancer · Virotherapeutics · Combination therapy

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Introduction

The progress in oncolytic virotherapy has emerged as potential therapeutic strategies. The aim of developing new therapies for the treatment of cancer is to design agents that have a large therapeutic index (i.e. high potency against established malignant cells with less native cytotoxicity). Therefore, the designed anticancer virotherapeutics should have potential to eliminate cancer cells while leaving normal cells intact.

Naturally occurring lytic viruses have evolved to infect, replicate in and lyse human cells. It is evident that the replication cycle of many viruses exploits the same cellular pathways that are altered in cancer cells [1, 2]. Recent advances in molecular biology of cancer, as well as the technologies to genetically engineer viruses, have led to the concept of oncolytic viruses.

Oncolytic Wild Viruses and Virotherapy

Some wild viruses with natural oncolytic activity in human tumors, like myxomaviruses, bovine herpesvirus 4, reovirus,

New castle disease virus (NDV), Coxsackie virus, Vesicular stomatitis virus (VSV), Parvoviruses, etc. produce unspecific infections in humans, and in some birds and mammals. These viruses are referred as 'Oncolytic Wild Viruses' and are under intense research for virotherapy, but their oncolytic efficacy has been limited in some preclinical and clinical assays and trials [3].

Oncolytic viruses are cancer therapeutics based on viruses whose replication is restricted to malignant cells [5–8]. In general this tumor selectivity can be achieved in one of two ways; (i) some viruses that normally do not cause disease in humans can nevertheless replicate in cancer cells; where the interferon (IFN) anti-viral response is frequently non-functional or in tumors (where an immunosuppressive environment exists). These are typically small viruses with fast replication cycles, such as reovirus [8, 9], Newcastle Disease Virus [10] or VSV [11]; (ii) the second group of oncolytic vectors are based on viruses that are either used as vaccines against common disease-causing viruses; such as vaccinia virus [12] or the Edmonton strain of measles virus [13] or on viruses that themselves cause known disease in humans; such as adenovirus [14], HSV [15] or poliovirus [16]. These tend to be larger viruses that are amenable to genetic engineering to produce or enhance their tumor selectivity. This increased selectivity is normally achieved through the deletion of viral virulence genes that are redundant for viral replication in tumor cells. As a result viral replication is attenuated in normal tissues, but proceeds normally in cancer cells. Because many of the alterations produced in a cancer cell during transformation are similar to the adaptations that a virus needs to induce in a cell for successful replication [17, 18], many such virulence genes exist (as witnessed by the oncogenic properties of some viral genes and the production of some cancers as a result of chronic viral infections). These viral gene products may fit into one of several different categories, including immune modulators (that are not required in the immunosuppressive tumor environment) [19]; anti-apoptotic proteins [4]; or inducers of cellular proliferation [20], meaning that different viral vectors (sometimes even produced from the same viral backbone) may target tumors based on unique or independent tumorogenic properties. Additional approaches to achieving tumor-selective replication of oncolytic viruses have also met with some success. These include the use of tissue or tumor specific promoters to drive expression of an essential viral gene [21–24]; and the alteration of viral-surface receptors to selectively target ligands that are highly expressed on tumor cells or in the tumor microenvironment [25–27].

One advantage of oncolytic viruses is that they are known to destroy tumors by several distinct mechanisms, which typically do not overlap with the mechanisms induced by traditional therapies [12, 28, 31]. In addition to directly destroying infected tumor cells as a result of infection (which also leads to amplification of viral copies within the tumor), many oncolytic vectors can induce a potent immune response within the tumor. This immune response can overcome localized immune suppression, and may even create an in situ vaccination effect through cross-presentation of tumor associated antigens to the host immune response. Furthermore several viruses have been demonstrated to induce a robust vascular collapse within the tumor that is capable of destroying further tumor cells [29, 32]. Because oncolytic viruses express their genomes primarily within the tumor and amplify the copy numbers of their genes in the tumor microenvironment, their effects can be enhanced through the expression of therapeutic transgenes [33, 34].

Oncolytic viruses acts not only for the design of simplistic infections of a tumor or its host, but rather the combination of two or more oncolytic viruses acting against different oncogenic cascades while suppressing antiviral immunity, allowing the viruses to act and enhancing the pre-existing but unexpressed antitumoral immunity of the host. The elements of this evasive anti-tumor immunity now reinforced, are to remain preserved in memory cells even after the rejection of the targeted tumor. There is order, and no "chaos," in the field of oncolytic virotherapy of human cancers. The problems are recognized, confronted, and resolved or, if for the time being unresolved, the gathering of better factual information is being persued for the resolution of the intricate unsettled questions. Oncolytic viruses have been used as an exciting new anticancer strategy with the potential to target both localized tumors and more advanced metastatic lesions [5, 30, 35].

Direct cell killing caused by viruses is an active and highly complex process involving many cellular pathways; hence the occurrence of drug resistance appears unlikely. Furthermore, additional mechanisms, such as the stimulation of the humoral and cellular immune response of the host could potentially enhance virus-induced tumor regression. Some viruses have been successfully engineered to encode specific epitopes capable of recognizing and killing cancer cells [36]. Increased therapeutic outcomes leading to immunotherapeutic strategy have recently been achieved by using oncolytic virus in combination with a potent immune agonist reagent [37].

Finally, by arming the oncolytic viruses with therapeutic genes and immune stimulating agent, their antitumor toxicity could be increased [4, 34, 37]. Efficacy might be improved by combining therapies with immune-stimulating agents [38, 39].

An oncogenic virus in one type of host; can be oncolytic when it replicates in another tumor in another species [40].

Strategies to Generate Tumor - Selective Viruses

There are several strategies that achieve tumor-selectivity of replication-competent viruses, some of which are discussed here:

- Inherent tumor - selectivity
- Attenuation of wild type virus through the targeted deletion of viral genes
- Transcriptional targeting
- Cellular targeting
- Escape from immune system

Inherent Tumor-Selectivity

Several RNA virus species are tumor-tropic, which is partly the result of their ability to grow exclusively in cells with defective antiviral response systems (e.g. Newcastle disease virus (NDV) and vesicular stomatitis virus (VSV) [41]). These RNA viruses are sensitive to inhibition by interferon, and thus normal cells are almost completely protected from infection and replication. Where as tumor cells lack functional interferon response, hence are rapidly lysed. Replication of reovirus, which is another RNA virus with inherent tumor-selectivity, is restricted by activation of the double-stranded RNA-activated protein kinase (PKR) by early viral transcripts [42].

Increased levels of Ras activity, as is frequently observed in a wide variety of human tumors, counteract this inhibition by activating a phosphatase that antagonizes the effects of PKR, which consequently enables virus replication [43–46].

The neurovirulence factor ICP34.5 has been characterized as an inhibitor of PKR. Therefore, PKR-induced shutoff of cellular protein synthesis following infection with HSV is circumvented by ICP34.5 [47].

Attenuation of Wild-Type Viruses Through the Targeted Deletion of Viral Genes

Attenuation - Restricting virus replication to malignant cells, which involves deletions of viral gene regions or entire genes that are dispensable in tumor cells, but are crucial for efficient replication in normal tissue. Genetically modified, tumor-selective mutants have been described for a variety of virus species, including herpes simplex viruses (HSV), adenoviruses, vaccinia viruses and polioviruses [48, 50, 52, 54]. Tumor cells and cells that have been infected by viruses exhibit significant similarities in their abilities to interfere with signal transduction pathways, for example, promoting the transition from the prereplication (G1) to replication (S) stage of the cell cycle because they generate deoxynucleoside triphosphates (dNTPs), which are needed for DNA synthesis [1, 2].

Several viral gene products interact with cellular components, and thereby influence the cell cycle and cell survival [1, 2]. A variety of mutants have been designed with functional inactivation of the viral genes that encode for thymidine kinase, ribonucleotide reductase and infected cell protein 34.5 (ICP34.5) (e.g. HSV1716, dlsptk and hrR3), to target HSV replication to malignant cells [48]. To reduce the probability of the occurrence of wild-type revertants, and to increase the safety level, some viruses contain multiple mutations within their genomes (e.g. G207) [55]. In addition to HSV, other tumor-selective adenoviruses generated using this approach has been reported [56].

Binding of the adenoviral early gene 1 A (E1A) proteins to the Rb protein, triggers the release of the E2F transcription factor which is important for regulation of expression of cellular genes that control cellular DNA synthesis and proliferation [57]. However, the uncontrolled release of E2F and entry of quiescent cells into the cell cycle induces the accumulation of active p53 in the nucleoplasm, which causes growth arrest or apoptosis before the virus can replicate productively [58]. Therefore, adenoviruses encode another set of proteins, the E1B proteins (E1B55K and E1B19K), that counteract the p53-mediated effects triggered by E1A [59–61]. The E1B55K-deficient adenovirus dl1520 (ONYX-015) is a promising anticancer agent [62–66]. Based on the attenuation approach, several oncolytic versions of vaccinia viruses that comprise mutations in the genes encoding thymidine kinase and/or vaccinia growth factor (VGF) render the viruses highly tumor-selective [52]. Poliovirus mutants selectively replicate in cell lines that are derived from human glioblastomas [53].

Transcriptional Targeting

The regulation of gene expression is influenced by various epigenetic mechanisms which involve transcriptional activation or inactivation. Tumor suppressor genes have been found to be epigenetically silenced leading to the pathogenesis of cancer. Therefore epigenetically silenced tumor suppressor genes might be a potential target in the treatment of cancer.

Transcriptional targeting is to restrict viral replication to malignant cells, involves the engineering of tumor or cell type specific promoters and enhancers into viruses to limit the expression of the genes that are essential for viral replication in tumors.

Replication-competent adenoviruses that have restricted expression of the E1A and E1B genes have been produced for prostate carcinomas using prostate-specific promoters, such as the prostate-specific antigen (PSA) promoter, the probasin promoter and combinations of both (e.g. CV706 and CG0787) [53–71]. Selective expression of E1A has been attempted in specific carcinomas, such as hepatocellular carcinoma (Alfa-fetoprotein promoter) and breast carcinoma (mucin-1 promoter and estrogen-receptor promoter) [22, 73–76]. In addition, the general characteristics of tumor cells (e.g. telomerase promoter and hypoxia-inducible factor responsive elements) have been used to design oncolytic adenoviruses [22, 76]. The expression of the essential immediate-early ICP4 gene, which is under control of the

albumin-enhancer-promoter, principally restricts replication of HSV to the liver and to hepatocellular carcinoma [77]. In a similar approach, the calponin promoter has been used to generate HSV mutants that replicate selectively in malignant human soft tissue and bone tumors.

Cellular Targeting

Cellular targeting is tumor-selective uptake of replication-competent viruses, can be achieved by modifications of the viral coat in a process. In theory, this strategy offers the advantage that more virulent viruses can be used; because these virus mutants would not infect normal cells, there should be a reduction in toxicity.

Colonic mucosa is continuously renewed by proliferating stem cells present at the base of the crypts. The onco-suppressive bacteriophages in the colon might be regarded as the battlefield where incipient tumor cells and bacteriophages compete for dominance. Accumulation of a large population of phage particles at colonic mucosa may have opportunity to transform stem cells. If phage particles get attached to and enter the inactive malignant stem cells and thus "xenogenize" them, a most powerful innate and adaptive host immune reaction could be mobilized and incipient colon cancers could be eliminated [78–82]. Phages are capable of binding tumor cells to inhibit tumor growth and proposed that the mechanism is mediated by the beta3 integrin signaling pathway [83–86]. Therefore oncolytic viruses could be designed by manipulating genetic components capable of transducing into inhabitant probiotic bacteria in enteric/urinary system for efficient expression and release of therapeutic proteins to trigger the beta3 integrin mediated signaling pathway. Oncolytic viruses have been successfully engineered to transduce specific cells by expressing epitopes that are recognized by particular cell-surface receptors and to express prodrug convertases and cytokines for use in cancer therapy [36]. Recently, synthetic viral particles have been designed that are capable of packaging therapeutic proteins, which can be released in a dose-dependent manner [87]. Similarly, protein-carrying viral nanoparticles have been used to deliver site-specific DNA recombinases, such as FLP, to precisely integrate or excise genetic components on the host chromosome [88]. They might also be used to deliver native or chimeric transcription factors that could transiently control the expression of target genes that are involved in therapeutic interventions, lineage control or induction of pluripotency [89].

Several attempts have been made, to modify the fiber proteins of Adenoviruses, in order to redirect the natural vector tropism away from normal cells towards malignant cells [90, 91]. More recently, HSV vectors with modified vector tropism have been produced. Engineered HSV-1 vectors have been designed that can only enter cells that express the interleukin-13 (IL-13) receptor, such as malignant brain tumors [92]. Blood borne Sindbis vectors specifically target and kill tumor cells [93].

Escape from the Immune System

The combination therapy has recently been emerged and supported the idea that the strategies which enhance immune activation against tumor-associated antigens can also be used to enhance the efficacy of virotherapy [94]. Incorporation of a tumor associated antigen within the oncolytic VSV have been reported to enhance the levels of activation of naive T cells against the antigen, which translated into increased antitumor therapy [94]. Oncolytic viruses influence cytolysis by multiple mechanisms including direct killing, cellular hypoxia resulting from the blockage of tumor vasculature, and release of inflammatory cytokine. More recent advancement towards virotherapy research is the combination therapy which has been proved to be increased survival of animal models [37, 95]. VSV is engineered to express a tumor antigen to increase the number of tumor-associated dendritic cells (DC) and tumor antigen presentation by combining VSV treatment with recombinant Fms-like tyrosine kinase 3 ligand (rFlt3L), a growth factor promoting the differentiation and proliferation of DC.

The receptor based T-cell activation and persistence, particularly for cytotoxic CD8þ T cells offer to design virotherapeutics to trigger anti-cancer response. The cellular receptor 4-1BB (CD137) is an effective agonist which is capable of triggering enhanced immune response against tumor cells when used alone or in combination with other antibodies [37, 96–98]. Some encouraging anti-tumor responses have recently been reported with limited efficacy in clinical trials [37]. Genetically engineered strain of oncolytic vaccinia virus has shown some encouraging anti-tumor responses with enhanced host immune responses [37]. Increased population of CD11b + and CD11c + myeloid cells in the tumor-draining lymph nodes, greater infiltration of CD8+ effector T and NK cells and a more sustained presence of neutrophils at the tumor site have been confirmed to be associated with tumor growth inhibition [37]. Vaccinia virus was combined with an agonist antibody (Ab) specific for the co-stimulatory molecule 4-1BB (CD137) for its capacity to elicit anti-tumor responses [37, 39].

In a recent study, Yu et al. systemically administered vaccinia virus mutants to mice and demonstrated that these mutants selectively enrich and amplify in gliomas, as well as prostate, bladder and metastatic mammary carcinomas [72]. The immune system is largely suppressed in tumors, micro-organisms that are distributed to a tumor escape the immuno-surveillance system of the host. The immune system of the host clears the remaining circulating viral particles shortly after intravenous delivery.

Clinical Trials with Oncolytic Viruses

As the replication of human viruses is typically restricted to human cells, initial testing began with intratumoral injection and proceeded to intracavitary (such as intraperitoneal) and intravascular administration (such as hepatic artery infusion). More recently, systemic (i.e. intravenous) applications have been studied.

Adenovirus

Wild-type adenoviruses were used as oncolytic agents in the middle 50s and the results of this protocol provide interesting considerations in terms of safety and efficacy for the current clinical trials with modified adenoviruses.

The most famous adenoviral therapy is ONYX-015 viral therapy. ONYX-015 is a manipulated adenovirus that lacks the viral E1B protein [99]. Without this protein, the virus is incapable of replicating in cells with a functioning p53 pathway. In most tumors, this pathway is deficient due to mutations, thus allowing ONYX-015 to replicate and lyse the cancer cells [62]. Tumors with an inactive p53 pathway had a better response [100]. In addition, ONYX-015 is currently being evaluated as a preventive treatment for precancerous oral tissue, since even in precancerous cells, p53 pathway-inactivating mutations will allow ONYX-015 to destroy and eliminate the precancerous cells before the tumor develops [99]. As a result of these promising *in vitro* and *in vivo* studies, ONYX-015 is now being tested for the treatment of a wide range of p53-deficient cancers in phase I and II clinical trials.

A genetically modified adenovirus named H101 by Shanghai Sunway Biotech gained regulatory approval in 2005 from China's State Food and Drug Administration (SFDA) for the treatment of head and neck cancer [103, 104]. Sunway's H101 and the very similar ONYX-015 have been engineered to remove a viral defense mechanism that interacts with a normal human gene p53, which is very frequently dysregulated in cancer cells [103]. These viruses do not specifically infect cancer cells, but they still kill cancer cells preferentially [103]. It appears to works best when injected directly into a tumor, and when any resulting fever is not suppressed [103]. Systemic therapy i.e. infusion through an intravenous line is desirable for treating metastatic disease [105].

Squamous Cell Carcinoma of Head and Neck (SCCHN)

The adenovirus ONYX-015 (also known as dl1520 and CI-1042) has made the most progress, and has proven to be a single safe agent in Phase I and II trials, for head & neck tumors which harbor p53 mutations [101, 106]. ONYX-015, combined with chemotherapy in phase II, produced an even better tumor response, leading to phase III trials [107]. Clinical responses were assessed and biopsies were obtained. ONYX-015 was injected intratumorally and treatment was well tolerated, with the main toxicity being mild flu-like symptoms (in particular, fever and chills). Viral replication was detected in 20 % of patients and, although an antitumor response was seen in 14 % of patients, a clinical benefit was not seen in the majority of the patients. However, when combined with chemotherapy, demonstrated an impressive clinical response rate in 63 % of the evaluated patients, with 27 % (eight patients) demonstrating full response to therapy (i.e. complete disappearance of all tumor manifestations) and 36 % indicating partial responses (i.e. tumor shrinkage by over 50 % of initial tumor volume) [108]. This response rate is far in excess of the expected response rates of patients that were heavily pretreated with chemotherapy alone [108]. At the end of 6 months, none of the responding tumors had progressed, whereas all non-injected tumors that were treated with chemotherapy alone had advanced. Tumor biopsy specimens obtained after treatment showed tumor-selective viral replication and necrosis induction [108].

Pancreatic Cancer

ONYX-015 was also administered intratumorally to patients with unresectable pancreatic cancer through computed tomography (CT)-guided injection [109] or endoscopic ultrasound injection in combination with intravenous gemcitabine [110] in a Phase I and Phase II clinical trials. The treatment was generally well tolerated. In the combination trial, two partial regressions and two minor responses were observed [110].

In some cancers with a wild-type p53 ONYX-015 actually did better than in their mutant p53 counterparts. These reports slowed the advancement through Phase III trials in the US, however recently China licensed ONYX-015 for therapeutic use as H101 [111]. TNFerada (a non replicating virus) failed a phase III trial for pancreatic cancer [111].

Premalignant Oral Dysplasia

ONYX-015 was used as local mouthwash therapy for premalignant oral dysplasia [99]. Histological resolution was seen in seven (37 %) out of 19 patients, but these effects were transient in the majority of patients.

Ovarian Cancer

Intracavitary applications of ONYX-015, a Phase I trial of intraperitoneal injection was undertaken in patients with recurrent and/or refractory ovarian cancer [112]. No significant toxicity was observed at the maximum dose. However, no clinical or radiographic evidence of a tumor response was observed in any of the patients [112].

Liver Metastasis from GI Tract Tumors: Intravascular Applications

Hepatic arterial infusion of ONYX-015 in combination with 5-fluorouracil (5-FU) and leukovorin for liver metastases of gastrointestinal (GI) tract tumors was tested in a combined Phase I and Phase II clinical trial [49, 51]. Although increased levels of liver enzymes and hyperbilirubinemia were transiently observed in a subset of patients, the regimen proved to be safe and feasible, with promising results. Delayed secondary peaks of viremia at 72 h post-inoculation were detected and interpreted as an indication of viral replication [49, 51].

Metastatic Solid Tumors and Metastatic Colorectal Cancer

To test systemic administration, ONYX-015 was given by intravenous infusion to patients with metastatic solid tumors (Phase I) [102], and in a Phase II clinical trial to patients with metastatic colorectal cancer [113]. Toxicity was manageable and consisted primarily of flu-like symptoms (including chills, rigors and fever); however. Viral DNA was detectable for as long as 72 h in 36 % of the patients, all of which developed neutralizing antibodies. Three out of the 18 treated patients had minor reductions of carcinoembryonic antigen (CEA) levels and seven patients were assessed as having stable disease for 11 to 18 weeks. However, a progression in disease was ultimately observed in all patients [113].

Prostate Cancer

Early clinical trials with adenoviruses that are transcriptionally targeted to replicate in prostate cancer cells have demonstrated promising results [21]. Intraprostatic injection of this virus CV706, caused mild flu-like symptoms and hematuria. However, 13 out of 20 patients (65 %) experienced a reduction in serum PSA of ≥30 % from pre-treatment levels & 50 % with highest dose [21]. Prostate biopsies revealed viral replication in individual patients [21]. The response rates were increased when the course of treatment was supplemented with radiation [114, 115].

Herpes Simplex Virus

Malignant Glioma

The HSV-1 variant G207 was tested to determine the safety of stereotactic inoculation for the treatment of recurrent malignant glioma [116]. Twenty-one patients were treated. There were no serious side effects and, importantly, no patient developed HSV encephalitis. In several individual patients, radiographic imaging suggested an antitumor response [116].

HSV1716 was administered by intratumoral injection after surgery, to two small groups of nine & twelve patients suffering from relapsed malignant glioma [117, 118]. The study demonstrated the therapeutic feasibility, with no signs of encephalitis. The data documented intratumoral replication within high-grade gliomas without causing toxicity in both HSV- seropositive and HSV- seronegative patients [118].

Metastatic Colorectal Cancer

Early clinical data suggested a safe toxicity profile for the virus NV1020, which is in ongoing combined Phase I and Phase II trials; as a therapy for liver metastases of colorectal cancer [119]. An oncolytic HSV-1 selectively destroys diffuse liver metastases from colonic cancer [120].

Using the "Direct evolution" methodology, have generated ColoAd1, a novel chimeric oncolytic virus. In vitro, this virus demonstrated increase in both potency and selectivity when compared to ONYX-015 on colon cancer cells. Furthermore, these results have validated this methodology as a new general approach for deriving clinically relevant, highly potent anticancer virotherapies [121].

Skin Metastasis of Solid Tumors

The immunostimulatory granulocyte-macrophage colony-stimulating factor (GM-CSF) is currently in Phase I clinical testing for skin metastases of solid tumors, but no clinical data has as yet been reported.

Metastatic Melanoma

HSV1716 was injected intralesionally into subcutaneous nodules of metastatic melanoma of five patients [122]. As an internal control, a second nodule was injected with sterile saline. A flattening of previously palpable tumor nodules and microscopic evidence of tumor necrosis were observed [122].

Newcastle Disease Virus

High Grade Glioma

MTH-68 and PV701 are two attenuated, non-recombinant strains of NDV, which is an avian paramyxovirus that causes flu-like symptoms in humans. Csatary et al. [123] examined the oncolytic potential of NDV by treating four cases of advanced high-grade glioma with daily intravenous injections of live attenuated NDV MTH-68 [123]. Two of the four patients had near complete disappearance of their gliomas, while the remaining two patients experienced stabilization of their disease. These data are nevertheless encouraging and provide grounds for further development [123, 124].

Squamous Cell Carcinoma

Intravenous administration of PV701 was tested in a large Phase I clinical trial as a single agent in 79 patients with advanced cancers [125]. Flu-like symptoms, fever and hypotension were recorded, but no serious side effects were observed. The majority of patients developed antibodies to NDV. After intravenous treatment, 14 patients had stable disease for 4 to 30 months, 7 demonstrated minor responses, 2 had partial response and 1 patient had complete response but then relapsed after 7 months [125].

Reovirus and Vaccinia Virus

Reovirus, an acronym for Respiratory Enteric Orphan virus, generally infects mammalian respiratory and bowel systems. Most people have been exposed to reovirus by adulthood; however, the infection does not typically produce symptoms. The link to the reovirus; oncolytic ability was established after it was discovered to reproduce well in various cancer cell lines and lyses these cells [126].

Reolysin is a formulation of reovirus that is currently in clinical trials for the treatment of various cancers [127]. No clinical data on the use of vaccinia virus (expressing the immunostimulatory GM-CSF protein) have been published.

Challenges of Oncolytic Virotherapy

Oncolytic viruses can rapidly replicate in and spread through 2D cell cultures that are derived from a variety of different tumor types. However, there are several factors that could hamper the efficient spread of oncolytic viruses within a solid tumor mass [128]. Physical barriers such as necrotic areas within the infected tumor, normal stroma cells and extracellular matrix, or the presence of the basal membrane, could limit the distribution and infection of the diffuse virus. Viral replication has underlined the importance of diffuse tumor inoculation for the control of tumor growth and for the initiation of a self-perpetuating process of intratumoral viral replication [129, 130]. In addition, the physical size of the administered virus particles and their interaction with the receptors that are present on normal cells could be crucial. New delivery technologies, such as convection-enhanced delivery (CED) of drugs to the brain, will need to be explored for oncolytic viral therapy of brain tumors to achieve an even virus distribution. CED enables potent drugs, which would otherwise be too toxic to the body, or drugs that are not capable of passing through the blood–brain barrier, to be slowly and continuously infused into particular brain tumors through small plastic catheters with infusion pumps, after surgery.

The immune system of the host could limit ongoing viral replication within the tumor, and rising antibody titers could neutralize repeatedly administered viruses before the tumor has been successfully eradicated [50].

The complement system might be another impediment to effective delivery via the intravenous route [131]. By contrast, immune responses could also enhance the efficacy of oncolytic viral therapy, where viruses that express immunostimulatory proteins [34, 132]. Cytotoxic T-cell responses that are directed against the tumor have been identified as a potentially important therapeutic factor [132]. Therefore, several methods that eliminate undesired immune effects while preserving beneficial properties have been proposed.

The production of neutralizing antibodies could be transiently ablated by administration of anti-CD20 antibodies (rituximab) against B-lymphocytes before oncolytic virotherapy. Alternatively, the exchange of blood plasma (plasma pheresis) will enable the elimination of antibodies that are directed against viruses from the bloodstream [128]. To prevent inactivation of administered viruses by the complement, the complement could be transiently neutralized by administration of cobra venom factor or cyclophosphamide (CPA) [133].

A major factor that could potentially lead to the rapid clearance of viruses from the bloodstream could be uptake into Kupffer cells, which are extremely active phagocytic cells that line the walls of the sinusoids of the liver [128]. However, preclinical experiments with several vectors have shown that systemic metastases can be targeted following intravenous administration, despite a level of clearance by the liver. Finally, sufficient expression of viral receptors on malignant cells is required for therapeutic efficacy [128] - a factor that has been identified as a potential limitation for oncolytic adenoviruses.

Activation of oncogenic signaling pathways that renders highly malignant cells less susceptible to therapy [134]. To overcome these prospective obstacles using inhibitors of the mitogen-activated protein kinase (MEK), this increases the expression of CAR on the cell surface and consequently restores the permissiveness of these cells to viral uptake [135].

Conclusion and Future Perspectives

The development of effective treatments for human solid tumors remains a significant challenge to cancer researchers and oncologists alike. This is due to the complexity of human solid tumors, with multiple, sometimes redundant, interacting signaling pathways [136], patient population differences [137], and the ability to acquire resistance to treatments including the newly developed targeted molecular therapies such as erlotinib, gefitinib, and imatinib [138]. Consequently, new agents, with unique mechanisms of action capable of confronting this complexity, are needed.

Naturally occurring or genetically engineered replication-competent oncolytic viruses, are new class of agents for the treatment of human cancer. The field of oncolytic viruses research can be divided into two overlapping disciplines. One approach uses recombinant DNA technology to engineer tumor selectivity into viruses primarily; these are human viruses such as adenovirus or herpes simplex virus. The alternative approach has been to rely on the inherent tumor selectivity that has been documented in a growing number of naturally oncolytic viruses [138–140].

The main obstacle for all these therapies is the human adaptive immune response, because antibodies invariably mobilize against the oncolytic viruses themselves. Can the viruses kill tumors faster than the body's immune system kills the viruses? "That's the big question," acknowledges Kirn. If the viruses fall short, giving drugs to suppress B cells, or using multiple different oncolytic viruses in succession, are possible solutions [103].

Potential hurdles have been identified, and their solutions are currently being explored in preclinical & clinical trials. To date, clinical experience indicates that these agents are safe. To increase potency, two key strategies are being pursued. Combination of oncolytic virotherapy with traditional chemotherapy and radiotherapy significantly enhances the efficacy of virotherapy, on a synergistic basis [141]. Oncolytic viruses are armed with therapeutic transgenes (e.g. prodrug-converting enzymes and antiangiogenic or immunomodulatory proteins) that induce bystander effects that are capable of eliminating tumor cells which are not directly killed through viral oncolysis [34]. The availability of systemic therapy in conjunction with oncolytic viruses will enhance the potential of oncolytic viruses to become a viable new therapeutic approach for the treatment of cancer.

Targeted therapy of cancer using oncolytic viruses has generated much interest over the past few years. In 2006, the world witnessed the first government-approved oncolytic virus for the treatment of head and neck cancer. Recent human oncolytic virus trials have shown consistent safety, with most unable to even reach the maximum tolerated dose [142]. Engineered oncolytic viruses with immune stimulating agents have opened a great hope to improve efficacy of targeted onco-virotherapeutics [37, 143].

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