Not all viruses are bad guys: the case for reovirus in cancer therapy

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Efforts to improve on cancer therapy have begun to capitalize on recent advances in our understanding of tumorigenesis. Tumor-specific characteristics are being exploited to develop selective antibodies and pharmacological inhibitors that specifically target cancer cells, and these agents are already showing clinical promise. None of these approaches, however, has captured our imagination as much as the use of replication-competent viruses to kill cancer cells. Whereas normal cells resist replication, tumor cells have an impaired antiviral response that sensitizes them to oncolytic viruses. One such virus is reovirus, a benign, naturally occurring virus that can effect tumor regression in animal models. Reovirus is demonstrating much promise in pre-clinical studies of cancer therapy and in clinical trials, where a lack of toxicity and signs of efficacy are generating excitement for this novel potential cancer therapeutic.

Despite advances in cancer screening and treatment allowing for overall declines in death rates, cancer remains an ever-present concern for our ageing population [1]. Current efforts to improve on cancer therapy are aiming to enhance drug efficacy while maintaining acceptable toxicity levels. To achieve this, novel therapeutics have been designed to target tumor-specific attributes to allow for higher doses with fewer side effects. For example, breast tumors overexpressing the epidermal growth factor (EGF) receptor family member, Her2, have been successfully treated using anti-Her2 antibodies [2]. Small molecule inhibitors have also been designed to target cancers; for example, through antagonism of estrogen activity in breast cancer (e.g. using Tamoxifen), or of the tyrosine kinase mutant protein, Bcr-Abl, in leukemias (e.g. using Gleevec; reviewed in [3] and [4]). Several replication-competent oncolytic viruses also take advantage of tumor-specific qualities to replicate within and specifically kill cancer cells. Herpes virus and adenovirus-based agents have been generated, which target actively cycling cells or cells with an inactive p53 response [5,6]. Replication competent virus therapy has the advantage that progeny virus production at the tumor site theoretically permits a greater therapeutic window.

Reovirus is another replication-competent, naturally occurring virus that preferentially kills tumor cells (Figure 1) [7]. First investigations into the mechanism behind tumor cell selectivity revealed that reovirus replicates preferentially in the presence of an active Ras signaling pathway, a common characteristic of cancer cells [8]. In addition to this antitumoral quality, reovirus is known for its benign pathology in humans; most adults have been infected, yet infections are subclinical (up to 100% of adults are seropositive [9]). The discovery of reovirus' oncolytic capacity, combined with its benign nature, has stimulated many pre-clinical studies and now clinical trials testing the feasibility of its use as an anticancer therapy [7,10–17]. This review will summarize recent advances made in studies of reovirus therapy of cancer.

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1359-6446/05/$ – see front matter PII: S1359-6446(05)03483-5

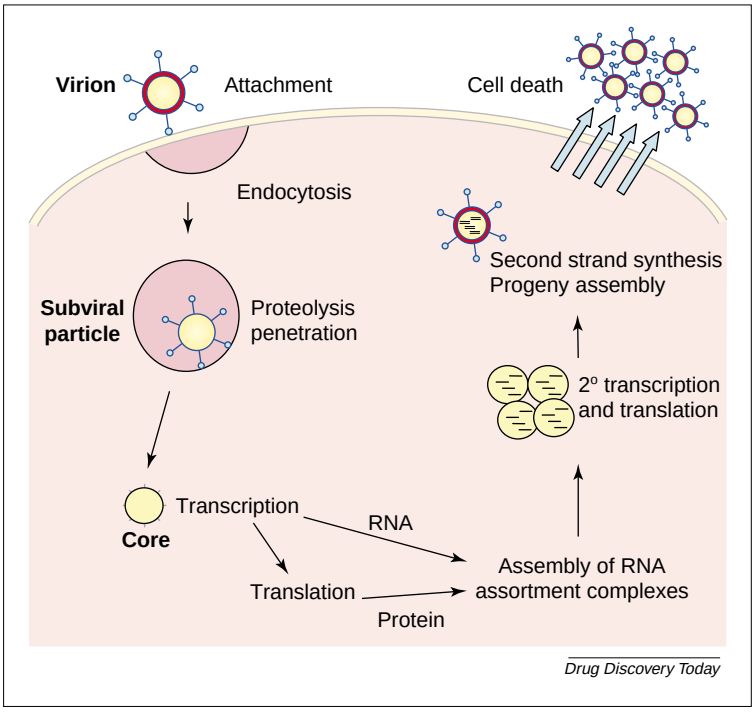

FIGURE 1

Overview of reovirus biology [60]. Reovirus is a small, non-enveloped, icosahedral virus found ubiquitously in the environment. It can be isolated from water sources, and shows a broad host range of infection. There are three serotypes of reovirus, with laboratory prototype viruses designated type 1 Lang, type 2 Jones, and two serotype 3 prototypes, type 3 Abney and type 3 Dearing. Type 3 Dearing is the strain that is currently being tested in clinical trials, and no modifications have been made to this isolate for cancer therapy. Reovirus has a double-stranded RNA genome present in ten discrete segments, which code for non-structural proteins involved in replication and pathogenesis, and structural proteins that make up the capsid. The viral life cycle begins with binding to the cellular receptors, sialic acid and junction adhesion molecule, followed by endocytosis [61,62]. Within the endosome, several changes in the outer capsid, including proteolysis, lead to capsid rearrangement and fusion with the endosomal membrane [63], which activates the viral RNA-dependent RNA polymerase. Notably, viral polymerase and capping reactions both take place within the capsid [64,65]. Following transcription and early translation, primary transcripts and protein products assemble to form RNA assortment complexes, which then carry out secondary transcription. During infection, reovirus can induce a G2/M cell cycle arrest in a manner dependent on reovirus σ1s protein [66]. Near the end of the replication cycle, viral factories gather (whose morphology varies with serotype and strain) and final assembly of virus then leads to host cell death and progeny release.

Reovirus and human disease

As mentioned above, reovirus is generally regarded as benign. Originally, the name reovirus was coined after its isolation from the respiratory and enteric tracts and its lack of association with a disease, and was hence designated an orphan virus [18]. There is substantial evidence supporting a lack of reovirus toxicity to adults, although there is some controversy over a role for reovirus infection in neonatal extrahepatic biliary atresia (EHBA). When infected with reovirus, newborn mice develop an unusual symptom first described over 50 years ago, called 'oily hair effect' (OHE), which resembles EHBA [19]. This occurs upon viral infection of bile duct epithelium, leading to inflammation, biliary obstruction, and ultimately resulting in lipid-rich stools that render the hair oily [20–22]. However, studies in humans attempting to associate neonatal EHBA with reovirus infection (detected by seropositivity or RT–PCR) have found conflicting results [23–30]. It has recently been proposed that results from studies measuring anti-reovirus antibodies could be confounded by infection with non-hepatotoxic serotypes of reovirus, leading to seropositivity in healthy individuals [21]. Given the ubiquity of reovirus infection, and the lack of reovirus toxicity in several investigations, it is possible that several factors such as serotype, timing of infection and patient immunological status determine whether infection leads to EHBA [21,30]. Overall, an etiological role for reovirus in EHBA remains under dispute.

Several studies have found that reovirus is nonpathogenic in adults. Early work by Rosen in 1963 examined reovirus infection in human volunteers at a correctional institution [31]. Reovirus infection was followed in the volunteers by assessment of seropositivity and fecal samples, and they found no symptoms associated with a productive reovirus infection. The safety of reovirus administration has also been shown in several animal cancer models. Generally, therapeutically active doses of reovirus in intratumoral, intracerebral and intravenous administration studies so far elicit little side effects in nude and immunocompetent animals [7,32,33]. Phase I and II human clinical trials have also demonstrated that intratumoral administration of reovirus is well tolerated in patients (see below). This lack of toxicity in cancer therapy models makes reovirus an attractive candidate for an antitumoral therapeutic.

Early indications of reovirus oncolytic potential

The first indications of reovirus' oncolytic potential emerged from studies of viral susceptibility of transformed and non-transformed cells. Hashiro *et al.* and Duncan *et al.* found that reovirus replicated well in several transformed cell lines but inefficiently in normal cells [34,35]; however, the underlying molecular basis of this susceptibility was unclear. It was not until investigations had progressed on the receptor for reovirus that it became evident that signaling within transformed cells dictated a suitable replication environment. Initial studies demonstrated that cells overexpressing the EGF receptor were highly susceptible to reovirus replication when compared with parental control cells [36]. Interestingly, reovirus binds the EGF receptor, suggesting that the virus was using the receptor to bind and infect cells [37]. Therefore, it was possible that this preferential replication in EGF receptor-overexpressing cells was a result of enhanced viral binding to the EGF receptor and entry. Alternatively, increased reovirus susceptibility can result from intracellular changes effected by the receptor. To test these possibilities, the susceptibility of cells expressing a truncated mutant lacking the receptor's extracellular domain was assessed; this oncogenic mutant of the EGF receptor is expressed by the retroviral v-*erbB* gene [38]. Remarkably, v-*erbB*-expressing cells displayed increased sensitivity to reovirus, demonstrating that signaling by the cytoplasmic domain of the receptor impacted on

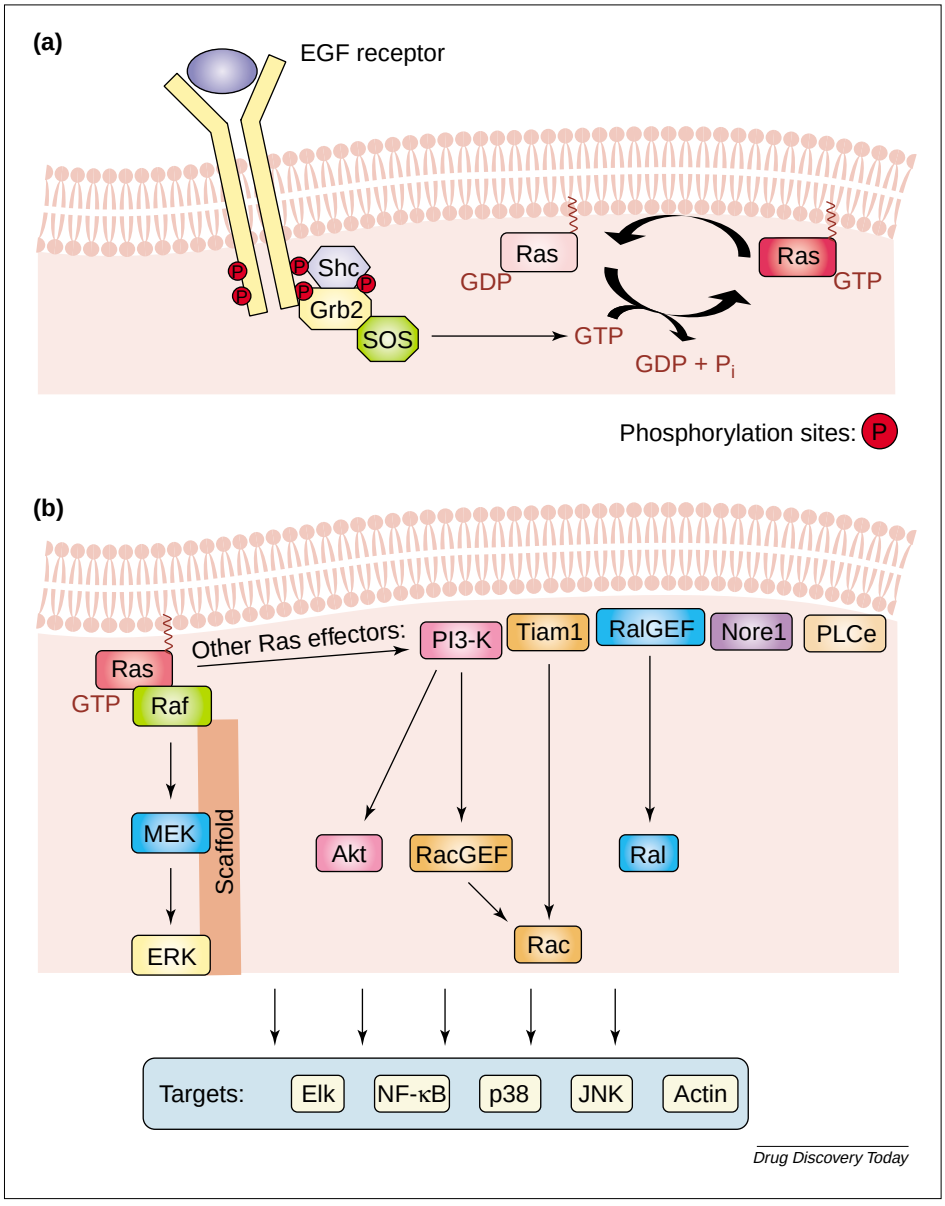

FIGURE 2

Ras signaling pathways downstream of EGFR. (a) Epidermal growth factor receptor activation of Ras. On binding to ligand, the epidermal growth factor receptor dimerizes and its tyrosine kinase activity is stimulated. Autophosphorylation within the receptor's cytoplasmic tail creates sites where effector molecules (such as Shc and Grb2) are recruited and phosphorylated. One mode of Ras stimulation is through recruitment and activation of Sos, which promotes the exchange of GTP for GDP on Ras. GTP-loaded Ras is then active to stimulate its own effectors. **(b)** Overview of Ras signaling. Formation of Ras-GTP leads to its activation, which subsequently promotes membrane recruitment and activation of >18 effectors (only six shown here). These effectors ultimately modulate the activity of diverse signaling pathways: transcription factors such as Elk1 and NF-κB, kinases such as p38 and JNK, as well as elements of the cytoskeleton (e.g. actin). The specific activation of individual effector pathways in normal cells is achieved by many means; for example, through the use of scaffolding proteins (shown here for Raf/MEK/ERK), which bring together elements of signaling modules to elicit defined cellular outcomes. Abbreviations: PLC, phospholipase C; GEF, guanine nucleotide exchange factor.

reovirus replication, rather than enhanced entry. This was further corroborated by the demonstration that viral entry and transcription still occurred in resistant cells.

Ras pathway signaling sensitizes cells to reovirus

To understand how the EGF receptor promoted reovirus replication, it was necessary to dissect downstream signaling in these susceptible cells. Normally, ligand-mediated activation of the EGF receptor leads to stimulation of signaling including the Ras pathway (Figure 2a). Ras is a small G protein that is active when bound to GTP and inactivated upon hydrolysis of GTP to GDP (reviewed in [39]). Ras-GTP promotes the recruitment and regulated activation of effectors at membranes (Figure 2b; reviewed in [40]). Oncogenic transformation occurs when regulation is lost, through constitutive activation of Ras by mutation or through aberrant activation of upstream proteins. Under these circumstances, uncontrolled Ras effector pathways act synergistically to promote tumorigenesis by driving stimulus-independent proliferation, anchorage-independent growth, enhanced survival and angiogenesis. Indeed, over 50% of human cancers demonstrate enhanced Ras pathway activation, emphasizing the potent effect of this signaling network on tumorigenesis.

As reovirus replication was promoted in EGF receptor-expressing and transformed cells, it was possible that active Ras contributed to viral replication. To test this, Strong *et al.* assessed reovirus replication in cells expressing an active mutant of Ras compared with that in parental NIH-3T3 cells, and found that Ras did promote replication [8]. Additionally, although viral entry and transcription took place in resistant, untransformed NIH-3T3 cells, these cells could abort viral infection. It was becoming evident that reovirus was a potential oncolytic agent that could be used to kill cancer cells selectively, whose genesis and survival often requires Ras pathway activation.

Reovirus therapy elicits tumor regression

First proof-of-principle studies for reovirus therapeutic activity were carried out in human cancer cell lines and a murine hind flank tumor model [7]. Initial experiments found that over 80% of cell lines originating from a variety of tumor types were sensitive to reovirus-mediated killing [7]. Work *in vivo* then tested reovirus therapy on human glioma xenografts in severe immunodeficient (SCID) mice. Coffey *et al.* found that 80% of tumors regressed after one intratumoral injection of reovirus. This was the first indication that reovirus-elicited cell killing could induce regression of an established tumor.

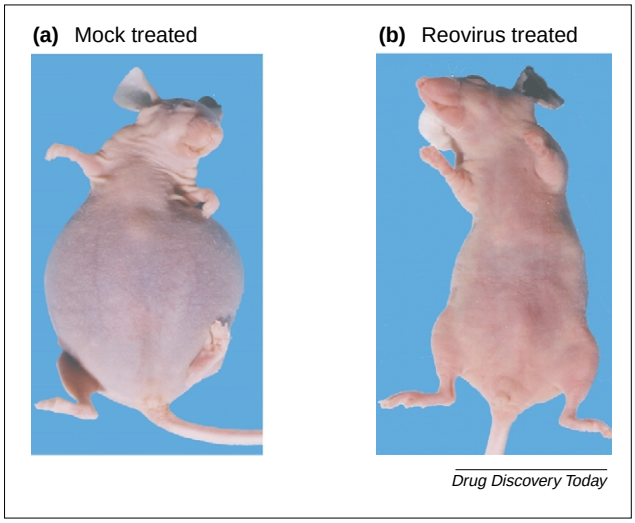

FIGURE 3

Reovirus treatment of human ovarian ascites tumor xenografts. Nude mice were injected intraperitoneally with MDAH2774 human ovarian tumor cells at day 0, followed by administration of live reovirus (**b**) or UV-inactivated virus [mock-treated (**a**)] at days 5 and 19. Photographs are of representative animals at day 28. Adapted from Hirasawa *et al.* [11] with permission.

A similar model was used to evaluate the effect of the immune system on reovirus therapy. Tumor allografts using Ras-transformed C3 cells in syngeneic, immunocompetent mice were treated with multiple injections of reovirus over several days. Intratumoral (i.t.) virus still elicited tumor shrinkage and led to complete remissions without reovirus-induced morbidity or mortality. These findings were substantiated by repeating the above experiment in mice previously immunized against reovirus [7]. As mentioned, most individuals harbor circulating anti-reovirus antibodies, which may hinder viral therapy [9]. Notably, it was found that a previously primed immune system could not prevent regression. Altogether, these studies pioneered investigations of reovirus use as an oncolytic agent, and ultimately led to clinical trials testing the feasibility of reovirus therapy in patients.

Reovirus therapy of solid malignancies

Several, more specific investigations into reovirus therapy of solid cancer types have since been undertaken. Overall, all studies demonstrate effective *in vitro* cell killing and/or cytopathic effects by reovirus in the majority of tumor cell lines tested. In the case of colon, ovarian and breast cancer, control cells derived from normal tissue resisted virus-induced cell killing [10,11]. A variety of *in vivo* rodent tumor models using tumor cell line implants have also been tested. Generally, in SCID mice, a single injection of reovirus induced tumor regression, and in immune competent animals, multiple injections were necessary for an anti-tumoral effect. Additionally, several *ex vivo* human tumor surgical specimen were used to confirm that viral replication was not limited to laboratory-propagated cell lines. To test human specimen, tumor tissue removed during surgery is immediately dissociated, infected with reovirus, and viral replication assessed in the laboratory. Some highlights of these studies are discussed below.

It is known that a large proportion of human colon cancers have a mutation in the K-*ras* proto-oncogene (reviewed in Ref. [41]), which makes them particularly suitable for potential reovirus therapy. Indeed, reovirus-induced cell killing was demonstrated in several human colon carcinoma cell lines, reflecting the prevalence of Ras activation, whereas a normal control cell line had low Ras activity and resisted reovirus [11]. These promising results were validated *in vivo*. Perhaps the most prevalent incidence of *ras* mutation in a form of human solid tumors is seen in adenocarcinoma of the exocrine pancreas, where up to 95% of tumors carry a K-*ras* mutation [41]. Correspondingly, pancreatic cancers are also sensitive to reovirus therapy in pre-clinical testing [16].

Elevated Ras activity is also common in malignant gliomas, thus these tumors, too, were attractive candidates for reovirus targeting [42]. Indeed, Wilcox *et al.* demonstrated reovirus-induced killing in 83% of malignant glioma cell lines tested. Reovirus treatment of orthotopic, intracerebral tumors in nude mice led to significant increases in survival, and further studies showed that reovirus is also effective against gliomas in the presence of a competent immune system [54]. Of note, not all sensitive cell lines had elevated Ras pathway activity, estimated by assessment of activation of the downstream element, MAPK. This might be explained by the fact that activation of non-MAPK Ras effector pathways can confer susceptibility [43]. It must be appreciated that the ultimate target(s) of Ras signaling that promote(s) reoviral oncolysis has yet to be defined before we can accurately choose a predictor of therapeutic effectiveness.

Another neurological tumor, medulloblastoma, was also evaluated for reovirus sensitivity [12]. Medulloblastoma is the most prevalent pediatric brain tumor, and because of its metastatic tendencies, it is difficult to treat without inducing severe neurological damage [44]. Although little was known regarding Ras activation in medulloblastoma, Yang *et al.* found that reovirus-mediated killing of cell lines coincided with elevated Ras activity [12]. They continued to demonstrate a remarkable more than doubling of survival time in an orthotopic cerebellar medulloblastoma model. These results clearly indicate that reovirus has promise in the treatment of neurological tumors of various origins.

Another orthotopic tumor model where reovirus has shown efficacy is in an ascites xenograft model of human ovarian cancer [11]. Mice with ascites tumors undergoing therapy maintained normal body weight and exhibited increased survival rates compared with mock-treated controls (Figure 3). In this form of cancer, high Ras activity could be a result of amplification of the EGF receptor family member, Her-2 [45]. Activation of the rat Her-2 homologue, Neu, sensitizes rodent cells to reovirus, thus this could

represent a contributing factor to ovarian carcinoma susceptibility (KL Norman *et al.*, unpublished).

Similar to ovarian cancer, amplification of Her-2 also occurs in up to one-third of breast tumors [45]. Her-2 expression in breast cancer correlates with poor prognosis and depends on Ras for transformation [45,46]. In addition, genetic aberrations in breast cancer that lead to anti-estrogen therapy resistance often result in upregulation of growth factor receptor signaling, which in turn activates Ras [47]. As these poor prognosticators predict a favorable environment for reovirus replication, our group tested reovirus efficacy against breast carcinoma [10]. Similar to other cancers, several cell lines were sensitive to reovirus, i.t. virus injection induced xenograft regression, and surgical specimens were able to replicate reovirus. Now that it is evident that ovarian and breast cancer are potential reovirus targets, further studies should address the contribution of growth factor signaling in human cells to viral susceptibility, and how current therapies such as Herceptin (Genentech, www.herceptin.com) and selective estrogen receptor modulators (SERM) might impact on reovirus efficacy.

An interesting *in vitro* model was recently employed to assess the selectivity of reovirus killing of bladder cancer (transitional cell carcinoma) cells [17]. Multicellular spheroids were first grown by co-culturing tumor cells with fibroblasts on semi-solid agar. Under these conditions, nontransformed fibroblasts grow within the centre of the spheroid whereas tumor cells line the periphery. Once spheroids were exposed to reovirus, the tumor cells were selectively killed, whereas fibroblasts remained viable. In this three-dimensional co-culture system, reovirus appears to maintain its oncolytic specificity, which has been confirmed *in vivo* in animal models of superficial bladder cancer [48].

Hematopoietic tumors and purging strategy

Although the majority of cancers tested derived from solid tissue are reovirus-sensitive, the first study of tumors of hematopoietic origin revealed a surprising proportion of reovirus-resistant samples. Alain *et al*. focused on reovirus therapy of lymphoid malignancies, examining various types of non-Hodgkin's lymphoma as well as B-cell chronic lymphocytic leukemia (B-CLL) [14]. Reovirus productively infected all diffuse large B cell non-Hodgkin's lymphomas (DLBCL) and less than half of Burkitt's lymphomas tested. When tested *in vivo*, tumors established from the reovirus-resistant Daudi Burkitt lymphoma cell line remained refractory to reovirus therapy, and those derived from the sensitive Raji cell line regressed in response to reovirus treatment. Numerous primary lymphoid cancer samples from patients were tested *ex vivo*, of which 100% of B-CLLs and 100% of DLBCLs were sensitive to reovirus [14]. The susceptibility of B-CLL and another DLBCL sample has also been shown by Thirukkumaran *et al*. [15]. Notably, five of six follicular lymphomas did not support reovirus replication.

The mechanism behind the differential sensitivities of lymphoid malignancies to reovirus oncolysis is not clear. Interestingly, interferon (IFN)-α therapy benefits patients with follicular lymphoma, suggesting that this cancer type maintains IFN sensitivity [49]. Because reovirus is inhibited by IFN signaling in some cell types, one could speculate that an intact IFN antiviral response might contribute to reovirus resistance in follicular lymphomas. It is also noteworthy that Ras signaling is activated once follicular lymphomas progress to DLBCL [50]. Whether Ras activation contributes to DLBCL sensitivity to reovirus remains to be explored. B-CLLs, however, are particularly resistant to the antiproliferative effects of IFN's, which might explain their overall susceptibility to reovirus infection [51]. It will be interesting to directly examine IFN's effect on reovirus oncolysis in hematopoietic tumors and use this insight to predict therapeutic effectiveness and improve on efficacy.

Studies have also looked at the sensitivity of normal human hematopoietic progenitors to reovirus killing [14,15] . It was found that exposure to reovirus did not significantly change CD34+ clonogenic potential and that granulocyte colony-stimulating factor (G-CSF)-stimulated stem cells were also not capable of supporting reovirus replication. These findings suggest that reovirus could be used to purge stem cell product of contaminating cancer cells before autotransplantation, without affecting the number or function of the normal cells. Current purging techniques leave contaminating cancer cells in up to 30% of autografts, and these are likely to contribute to cancer relapse following transplantation [52]. With this in mind, Thirukkumaran *et al*. tested whether cancer cells could be selectively killed by reovirus in the context of human stem cells [15]. They found that reovirus could successfully purge hematopoietic progenitor cells of contaminating monocytic and myeloma cancer cell lines, as well as several *ex vivo* human samples of lymphoma, myeloma and Waldenström macroglobulinemia, with no changes in stem cell numbers. However, one Burkitt lymphoma and one follicular lymphoma resisted reovirus treatment [15]. There is substantial evidence that not only are normal human stem cells resistant to reovirus, but this property can be exploited to utilize reovirus as an agent to purge residual disease before autotransplantation. Further studies will evaluate the effects of viral infusion back into the patient and possible countermeasures to potential viral toxicity associated with i.v. delivery [15].

Metastatic tumor therapy with reovirus

As metastasis is the major cause of death in cancer patients, systemic delivery of reovirus to remote tumor sites marks an important milestone in its development as an oncolytic agent. The first indications that reovirus could be used to treat established or inhibit metastases came from xenograft models of human cancer in immunocompromised animals [10,12,14,33]. Xenografts of a human breast cancer cell line were inoculated over both the left and right hind flanks

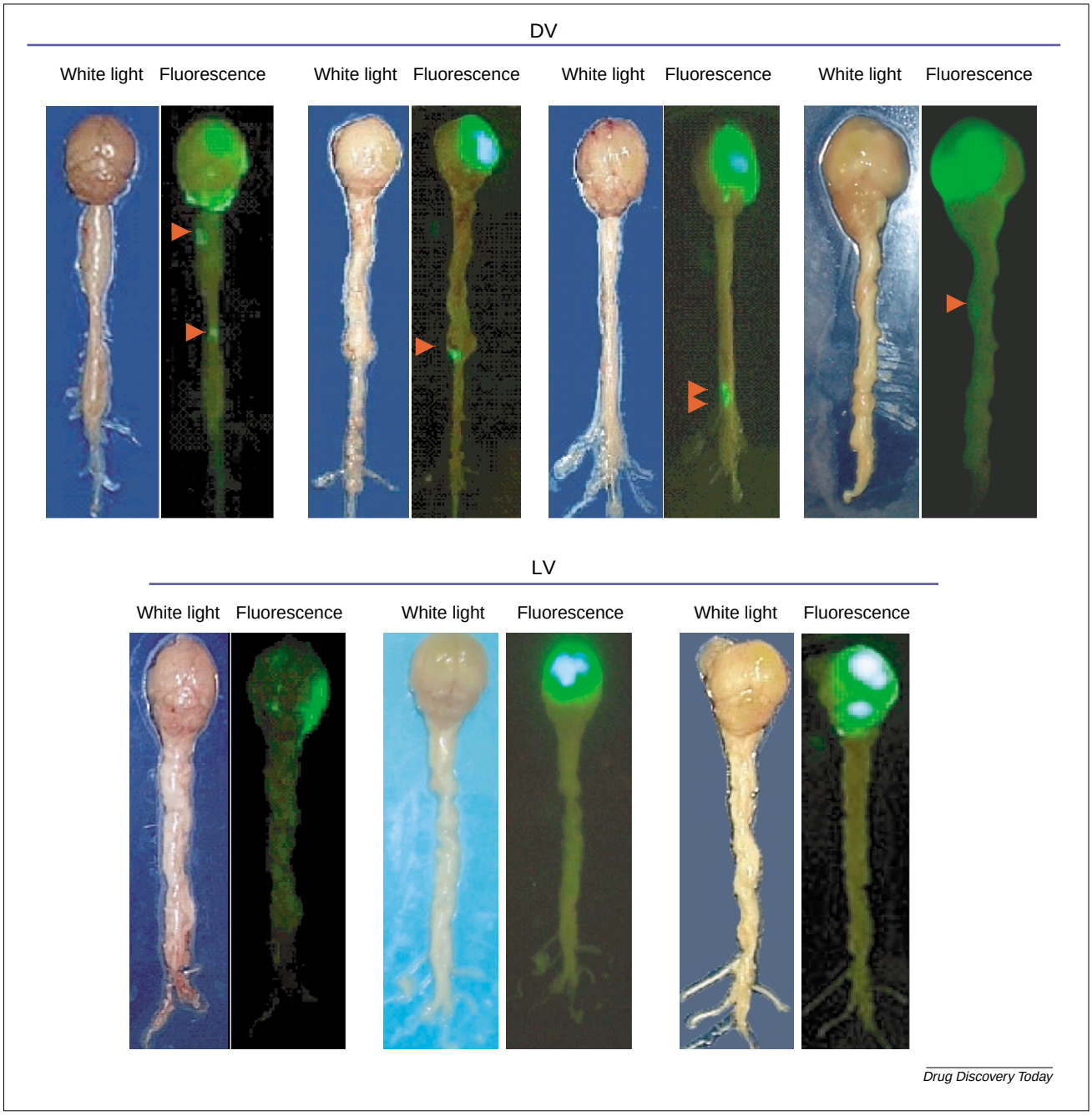

FIGURE 4

Reovirus treatment of medulloblastoma in mice. Human Daoy cells were transfected with GFP for visualization of tumors *in vivo* and then inoculated into the right putamen of mice. Mice were given multiple injections of live virus (LV) or were mock treated with UV-inactivated, dead virus (DV). Photographs depict white light images and fluorescent images of dissected brain and spinal cord. GFP-containing Daoy cells in spinal cord metastases (green) are indicated by red arrows and were found in all of the DV- but none of the LV-treated group. Adapted from Yang *et al.* [12] with permission.

and reovirus was injected into only one flank tumor [10]. Reovirus replication and regression was observed at both injected and noninjected contralateral sites. In another model, tumors derived from the Raji lymphoma cell line were treated with i.v. reovirus [14]. Again, remote tumor growth was suppressed in live virus-treated mice.

Two studies have shown inhibition of invasion and metastasis from a primary neurological tumor during reovirus therapy [12,33]. First, in a medulloblastoma model, intracranial tumors in nude mice were given multiple i.t. reovirus injections. As expected, all dead-virus-treated control animals developed spinal cord or leptomeningal metastases. None of the live virus-treated animals, however, had detectable metastases (Figure 4). Yang *et al.* have postulated that, as part of the metastatic program, tumor cells select for high Ras activity and thus present favorable targets for reovirus, an interesting hypothesis that remains to be tested.

In the second study, the ability of reovirus to treat breast tumors in the brain was evaluated [33]. Intracranial

reovirus administration prolonged animal survival in this model and prevented local tumor invasion and metastasis. Notably, reovirus did not cause mortality when administered intracranially up to doses of $1x10^8$ plaque forming units (PFU) in the nude mice used in this study. The lack of significant toxicity was reflected in the mild histological changes in the brain on reovirus injection and absence of learning and behavioral deficiencies. These studies suggest that reovirus therapy might prevent local invasion and metastatic tumor spread.

The next challenge was to address evading immune inactivation of circulating reovirus without increasing toxicity to the individual. A comprehensive study addressing the potential of i.v. reovirus administration in immunocompetent murine models of metastatic cancer [32] examined two types: localized, subcutaneous tumors and diffuse tumors disseminated in the lung. In both models, reovirus was administered intravenously by tail vein injection. Reovirus treatment suppressed tumor growth and improved animal survival in localized (subcutaneous) and diffuse (lung) metastatic cancer models. Viral replication was evident in tumors, confirming that the virus could target remote tumors and amplify at the tumor site. This also suggests that tumor growth suppression was not solely an immune-mediated effect.

Although growth suppression was initially evident in the localized subcutaneous model, after three weeks of i.v. reovirus therapy, tumors resumed growing. This coincided with rising anti-reovirus antibody titers in the blood, suggesting that an immune response to reovirus was compromising i.v. therapy. This was also observed in a similar model, where mice were immunized against reovirus before tumor establishment and treatment [32]. When animals were primed against reovirus, very little therapeutic effect was achieved by i.v. reovirus administration. However, when reovirus therapy was combined with the immunosuppressants cyclosporine A, cyclophosphamide or anti-T-cell antibodies, therapeutic efficacy was prolonged and enhanced (Figure 5). Together, these data suggest that not only is i.v. reovirus therapy of tumors feasible in terms of toxicity and efficacy in murine models, but also that antiviral immunity can be overcome with immunosuppressant administration.

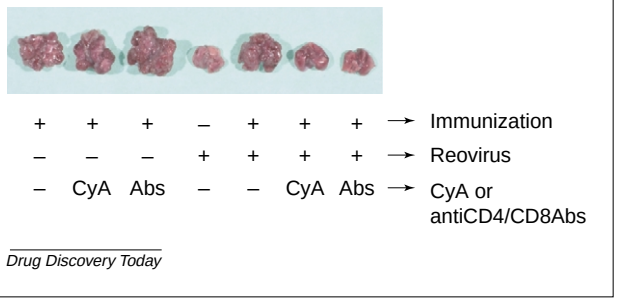

FIGURE 5

Effect of immune suppression on reovirus treatment of metastatic tumors in immunocompetent mice. C57BL mice were immunized with reovirus two weeks before the experiment. At day 0, Lewis lung carcinoma cells were inoculated intravenously to colonize the lung. At day 7, 13 and 19, mice were treated or mock treated intravenously with reovirus in the presence of the immunosuppressants Cyclosporin A (CyA) or anti-T cell antibodies (Abs). Photographs indicate overall lung size under various treatment regimens. Adapted from Hirasawa *et al.* [32] with permission.

Human clinical trials with reovirus

So far, one Phase I study and one Phase II study examining reovirus therapy in cancer patients have been completed. The Phase I study tested the safety of intratumoral reovirus administration in patients who had exhausted all conventional cancer treatments and who had accessible, subcutaneous tumors [53]. Dose escalation up to 10^{10} PFU of reovirus resulted in no serious adverse events and thus, no dose-limiting toxicities were found. As a secondary endpoint in the study, tumor responses were also evaluated. 61% of patients demonstrated some response to viral therapy, with a range of 32–100% tumor regression being observed. Additionally, evidence of noninjected, remote tumor changes was documented. This has led to a Phase II trial examining viral activity in reovirus-treated human prostate cancer (www.oncolyticsbiotech.com). The trial consisted of patients who were given one intraprostatic injection of reovirus, followed by prostatectomy after approximately three weeks. Histopathological analysis of the prostate gland indicated that apoptotic tumor cell death occurred in 4 of 6 patients. An additional patient exhibited a 53% drop in PSA level and 67% decrease in prostate size before prostatectomy. The final patient demonstrated no signs of reovirus activity. Again, no concerns of toxicity were raised in this second trial, and histopathological analysis suggested that reovirus specifically elicited tumor cell death while adjacent normal tissue remained unaffected. This was supported by the finding that immune cell infiltration was restricted to the tumor and was not observed in normal tissue.

A Phase I glioma trial has also been initiated in light of preclinical results demonstrating that intracerebral reovirus administration to cynomologous monkeys is well tolerated [54]. The main objective of this clinical trial is to assess the safety of reovirus administration to patients with recurrent malignant glioma. Interim results so far indicate that one intratumoral injection of reovirus is well tolerated. A second ongoing Phase I trial is examining the safety of systemic delivery of reovirus however no results have yet been released.

It has not been reported for these trials that patient cohorts have been stratified according to Ras activity, nor has viral efficacy been analyzed with respect to Ras activity. This is likely due to the fact that it remains unclear what is the best predictor of reovirus efficacy; for example, as described above, reovirus can replicate in cells where Ras pathway signaling is activated independently of Ras itself. Altogether, intratumoral administration of reovirus appears to be safe and is already showing signs of efficacy in clinical trials.

Future directions

Despite much promise in preclinical and clinical studies, reovirus therapy could be improved in some aspects, for example through more efficient tumor killing, or through the selective therapy of patients predicted to respond to reovirus. There are several avenues of investigation that could be followed to maximize potential benefits of reovirus therapy.

To improve on efficacy, other oncolytic viruses have been genetically engineered. These modifications are intended to promote cell killing, augment specificity and stimulate lasting anti-tumoral immunity. Because of its double-stranded RNA genome and the tight packaging constraints imposed on progeny virus, genetic manipulation of reovirus remains a challenge. Modification of reovirus using a reverse genetics system has been demonstrated [55], however the application of this technology to improvement of reovirus cancer therapy has not yet been explored.

The study of reovirus interaction with current cancer therapies will be a necessary step in its therapeutic development. Combination with other therapies might promote susceptibility of tumor cells, as well as kill reovirus-resistant cells within a heterogeneous tumor. Some insight can be gleaned from engineered adenovirus studies, such as where viral therapy combined with traditional therapy have yielded promising results [56,57]. In these trials, adenoviral therapy synergized with chemotherapy to yield better tumor response rates and longer times to tumor progression. Given these results it will be interesting to find whether reovirus can also synergize with chemotherapeutics. Additionally, it will be very interesting to determine whether combination therapy with multiple oncolytic viruses will improve tumor responses. Given that oncolytic viruses act through differing mechanisms, it is likely that tumors (or subsets of cells within a tumor) resistant to one virus will be sensitive to other viruses. Moreover, antiviral immunity inhibiting one oncolytic virus type could be overcome by using alternative viral therapies.

As mentioned above, reovirus' oncolytic mechanism of action must be further defined. One recent study using a mutagenesis screen has uncovered 151 candidate genes affecting reovirus replication [58]. In another study, oncogenic Ras was found to promote reovirus replication through a Ral guanine nucleotide exchange factor (GEF) pathway [43]. These results are promising in light of literature documenting the importance of RalGEF signaling in cancer [59]. Also, use of selective pharmacological inhibitors indicates that the stress-activated protein kinase, p38, promotes reovirus oncolysis [43]. It will be interesting to determine whether stress signaling activated by chemotherapy and radiotherapy affects reovirus oncolysis. Finally, one group has documented the promotion of reovirus replication by pre-treatment of the virus with protease [67]. Protease pre-treatment might improve on virus delivery within intratumoral injections by promoting entry. Finally, although signaling dictating reovirus oncolysis has been examined extensively in rodent cells, further study of reovirus mechanism is essential in human cells. Overall, understanding the oncolytic capacity of reovirus will allow for its more effective use in the clinic.

Concluding remarks

Reovirus' benign properties combined with its oncolytic nature make it an ideal candidate as a novel cancer therapeutic. Several studies have now been published establishing that reovirus can replicate in human tumor cell lines of various origin, as well as effect tumor regression in animal models. Notably, recent studies in rodents have found that reovirus can be administered intravenously to treat remote tumors in an immune competent setting, and that suppression of the immune response can improve on i.v. therapy. If the potential problem of immune response can be avoided in a similar manner in humans, this will provide the opportunity to treat tumors normally unressectable by surgery. Overall, these studies have bolstered efforts to test reovirus therapy in a clinical setting, where promising toxicity profiles and early signs of efficacy are emerging.

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