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REVIEW

Reovirus and Tumor Oncolysis

Manbok Kim[1], Young-Hwa Chung[2], and Randal N. Johnston[1]*

[1]*Department of Biochemistry and Molecular Biology Faculty of Medicine, University of Calgary 3330 Hospital Dr NW, Calgary, Alberta, T2N 4N1 Canada*
[2]*Department Nanomedical Engineering Pusan National University 50 Chenonghak-ri, Samnangjin-eup, Miryang City 627-706, Republic of Korea*

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REOviruses (Respiratory Enteric Orphan viruses) are ubiquitous, non-enveloped viruses containing 10 segments of double-stranded RNA (dsRNA) as their genome. They are common isolates of the respiratory and gastrointestinal tract of humans but are not associated with severe disease and are therefore considered relatively benign. An intriguing characteristic of reovirus is its innate oncolytic potential, which is linked to the transformed state of the cell. When immortalized cells are transfected *in vitro* with activated oncogenes such as Ras, Sos, v-erbB, or c-myc, they became susceptible to reovirus infection and subsequent cellular lysis, indicating that oncogene signaling pathways are exploited by reovirus. This observation has led to the use of the virus in clinical trials as an anti-cancer agent against oncogenic tumors. In addition to the exploitation of oncogene signaling, reovirus may further utilize host immune responses to enhance its anti-tumor activity *in vivo* due to its innate interferon induction ability. Reovirus is, however, not entirely benign to immunocompromised animal models. Reovirus causes so-called "black feet syndrome" in immunodeficient mice and can also harm neonatal animals. Because cancer patients often undergo immunosuppression due to heavy chemo/radiation-treatments or advanced tumor progression, this pathogenic response may be a hurdle in virus-based anticancer therapies. However, a genetically attenuated reovirus variant derived from persistent reovirus infection of cells *in vitro* is able to exert potent anti-tumor activity with significantly reduced viral pathogenesis in immunocompromised animals. Importantly, in this instance the attenuated reovirus maintains its oncolytic potential while significantly reducing viral pathogenesis *in vivo*.

Keywords: reovirus, persistent infection, viral attenuation, viral oncolysis, oncogene signaling, viral pathogenesis

REOviruses (Respiratory Enteric Orphan viruses) are cytoplasmically replicating viruses comprised of two concentric protein capsids surrounding a genome consisting of 10 segments of double-stranded (ds) RNA (Nibert and Schiff, 2001). Each dsRNA segment encodes a single protein, except for the S1 gene segment, which is bicistronic. Reoviruses are ubiquitous viruses that have been isolated from a wide variety of mammalian species including humans. In humans, reoviruses are commonly isolated from the respiratory and gastrointestinal tract but they are not associated with any known diseases and are therefore considered to be benign (Tyler, 2001). Studies of human volunteers at a correctional institution in the early 1960's led to the conclusion that reoviruses possibly play an etiologic role in the generation of some minor respiratory/enteric illnesses, but in general reovirus infections are asymptomatic (Rosen *et al.*, 1963). Thus they were initially classified as orphan viruses, indicating a virus that is not associated with any known severe human disease.

* To whom correspondence should be addressed.
(Tel) 1-403-220-8692; (Fax) 1-403-283-8727
(E-mail) RNJohnst@UCalgary.ca

There are three serotypes of reovirus, based on their hemagglutination activity. Prototypical laboratory strains of each serotype were isolated from children's respiratory and enteric tracts and are designated Type 1 Lang, Type 2 Jones, Type 3 Abney, and Type 3 Dearing. All three serotypes of reovirus are found ubiquitously in the environment, including such sources as water and sewage. This, combined with the fact that reovirus possesses a highly stable unenveloped icosahedral capsid, explains why as many as 50% of adults aged 20-30 years have been exposed to reovirus over the course of their lives and thus carry antibodies against the virus (Jackson *et al.*, 1973). Seropositivity has been documented to be as high as 70-100% of subjects in some studies (Minuk *et al.*, 1985; Minuk *et al.*, 1987), despite the fact that most reovirus infections go unnoticed.

Innate oncolytic potential by reovirus

The most intriguing characteristic of reovirus is its innate oncolytic potential. Hashiro *et al.* (1977) were able to demonstrate that certain virally and spontaneously transformed cell lines of murine origin were susceptible to reovirus infection, whereas normal human and subhuman primate cells, primary mouse cells, normal rat kidney cells and baby ham-

ster kidney cells were not. Duncan *et al.* (1978) also found that normal and SV40-transformed WI-38 cells exhibited different sensitivities to reovirus infection, with cytopathology observed only in the transformed cells and not in normal cells, which nonetheless produced virus for a sustained period. Collectively, these observations suggested that reovirus infection efficiency is somehow linked to the transformed state of the cell. To further support this, when immortalized cells (which were non-tumorigenic *in vivo*) were transfected with oncogenes such as Ras, Sos, v-erbB, and c-myc, they became susceptible to reovirus infection (Strong and Lee, 1996; Strong *et al.*, 1998; Egan *et al.*, 2003). This indicates that oncogenic Ras and other signaling pathways can be exploited by reovirus. The underlying basis of preferential reoviral tropism in transformed cells is currently thought to be the presence of a defective cellular anti-viral response (PKR, dsRNA activated protein kinase) that is triggered in Ras-pathway transformed cells (Strong *et al.*, 1998). Because activating mutations of the proto-oncogene Ras occur in about 30% of all human tumors (Bos, 1989), for example in pancreatic (90%), sporadic colorectal (50%), and lung (40%) carcinomas and myeloid leukemia (30%), this observation has led to the use of the virus in clinical trials as a powerful anti-cancer agent against Ras oncogenic tumors (Norman and Lee, 2005). In addition to the exploitation of oncogene signaling, reovirus may also activate the host immune system to enhance anti- tumor activity. Because reovirus, a double-stranded RNA virus, is an efficient inducer of type I interferon, it is likely that a host-interferon response also plays an important role in reoviral oncolysis *in vivo* (Steele and Cox, 1995; Steele and Hauser, 2005).

Co-adaptation of virus and host during reoviral oncolysis

Because cellular transformation may arise through a variety of pathways, and because acquired resistance to other cancer therapeutic agents is commonly observed *in vitro* and *in vivo*, one can speculate that resistance to reoviral oncolysis in Ras-transformed cells might also arise in some cases. We recently (Kim *et al.*, 2007a) were able to show that a human fibrosarcoma cell line that contains a well-defined activating mutation in Ras can indeed acquire resistance to reovirus. The resistance to reovirus is associated with persistent infection and viral and cellular changes. Virus resistant cells (HTR1 cells, Kim *et al.*, 2007a) show a persistent low-level infection with reovirus and a significant reduction of endosomal cathepsin B activity, which is required for efficient reoviral entry into cells (Ebert *et al.*, 2004). Interestingly, the persistently infecting reovirus has also undergone a viral attenuation event (Kim *et al.*, 2007c).

For such persistent infections to be maintained, interactions between virus and cell should be modulated during long-term persistent culture such that a less cytopathic virus-host relationship would be established (reviewed in Dermody, 1998). Consistent with this, various types of viral and cellular changes undergone during reovirus persistent infection have previously been observed by many reovirus investigators (Ahmed *et al.*, 1981; Baer *et al.*, 1999; Ebert *et al.*, 2004). As shown in Fig. 1, persistently infected HTR1 cells (Kim, 2005; Kim *et al.*, 2007a) were able to maintain cellular division even in the presence of abundant viral particles. Interestingly, the HTR1 cells showed asymmetric distribution of intracellular viral factories (Fig. 1), suggesting that persistent infection of HTR1 cultures can be maintained for a long period of time (Kim *et al.*, 2007a). Thus, a co-adaptation process has evidently occurred, allowing cells and virus to bypass reoviral oncolysis *in vitro*. However, unlike resistance to chemo/radiation treatments, the acquired resistant cells established during reovirus infection were no longer tumorigenic *in vivo* (Alain *et al.*, 2006; Kim *et al.*, 2007a). Because persistent viral infection affects cellular physiology, it is likely that the acquired-resistance cells were still vulnerable to host immune activity (Kim *et al.*, 2007a). Taken together, acquisition of resistance to reoviral oncolysis *in vitro* appears not to affect reovirus oncolytic potential *in vivo*.

Reovirus pathogenesis in immune compromised hosts

Initially classified as an orphan virus, the reovirus is, however, not entirely benign in animal models. Several recent studies showed that reovirus caused so-called "black feet syndrome" in immunocompromised animals (Loken *et al.*, 2004; Kim *et al.*, 2007c). Because cancer patients often undergo immune suppression due to heavy chemo/radiation-treatments or advanced tumor progression, it is possible that reoviral pathogenicity could be a hurdle in anticancer

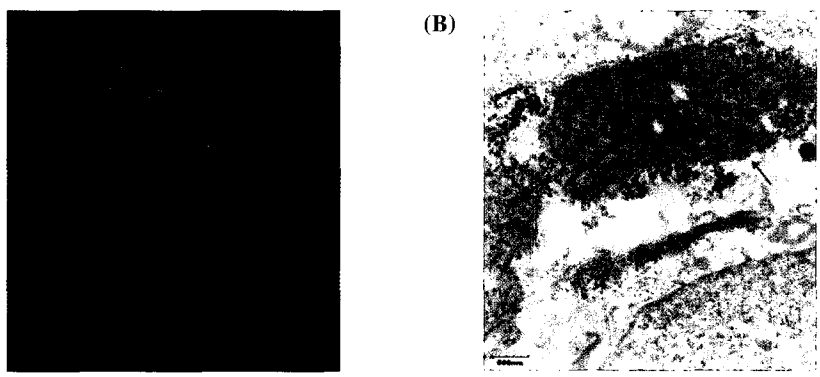

Fig. 1. Deconvolution confocal and electron microscopy of HTR1 cells. (A) Immunofluorescence staining of viral inclusion bodies in HTR1 cells (Green: indirect FITC immunostaining using reovirus antiserum. Blue: DAPI nuclear staining). (B) The presence of viral particles in cytoplasmic viral factories (arrow) where assembly occurs is confirmed by transmission electron microscopy.

therapy. Wild type reoviral tropism is not strictly limited to cancer cells and naturally occurring reoviruses may not be clinically innocuous, with animal models (immunodeficient or very young animals) revealing reoviral infection of cardiac myocytes (Terheggen *et al.*, 2003; DeBiasi *et al.*, 2004; Loken *et al.*, 2004; Kim *et al.*, 2007a) and reoviral induction of undesirable phenomena such as hemorrhage, fibrosis, hepatitis, pancreatitis, necrotizing encephalitis, and myocarditis (Sabin, 1959; Weiner *et al.*, 1977; Baty and Sherry, 1993; Richardson *et al.*, 1994; Mann *et al.*, 2002; Jun and Yoon, 2003; Loken *et al.*, 2004). Wild type reovirus also adversely affects development of rat and murine embryos, retarding development and inhibiting blastocytst formation (Heggie and Gaddis, 1979; Priscott, 1983). The absence of such pathogenic responses in healthy immunocompetent hosts suggests that adaptive immune responses may be critical in limiting reoviral pathogenesis in normal hosts. Using genetic assortment approaches, it was previously shown that the S1 gene of reovirus type 3 is not only an important viral antigen, but also a major determinant in reovirus-induced pathogenesis (Weiner *et al.*, 1977; Weiner *et al.*, 1980; Dichter and Weiner, 1984; Haller *et al.*, 1995).

Innate oncolytic potential by attenuated reovirus

Because of the pathogenic potential of wild type reovirus, especially in immunocompromised hosts as mentioned above, clearly there is an opportunity to develop strategies to modify the naturally occurring reovirus so that it can exert more selective viral oncolysis. Because the S1 gene segment of reovirus significantly contributes to virus-induced pathogenesis (Weiner *et al.*, 1977; Weiner *et al.*, 1980; Dichter *et al.*, 1984; Haller *et al.*, 1995), genetic attenuation of S1 may be expected to alter viral pathogenesis. However, unlike other DNA or single stranded RNA viruses, conventional genetic modification of reovirus is currently not possible due to its unique genetic composition (10 segmented double stranded RNAs) (Russell, 2002). Interestingly, persistent reoviral infection of cultured cells often leads to a genetic modification of reovirus by co-adaptation of virus and host (Ahmed *et al.*, 1981; Dermody, 1998). It has been speculated that interactions between virus and cell should be modulated during long-term persistent culture such that a less cytopathic virus-host relationship would be established (reviewed in Dermody, 1998).

Indeed, we recently found (Kim *et al.*, 2007c) that a persistently infecting reovirus derived from human fibrosacoma cells contained S1 gene mutations, including a premature stop codon mutation. Remarkably, the viral pathogenesis of reovirus expressing the truncated S1 coding sequence is significantly reduced in immunocompromised animal hosts while its oncolytic potential is retained (Kim, 2005; Kim *et al.*, 2007c; see Fig. 2). This is consistent with the S1-mediated viral pathogenesis as previously described. Importantly, the S1 attenuated reovirus does not affect murine embryonic stem cells' developmental potential whereas wild type reovirus blocks or eliminates stem cells *in vitro* or after transplantation *in vivo* (Kim *et al.*, 2007b). We also found that persistently infected cells derived from reovirus infected

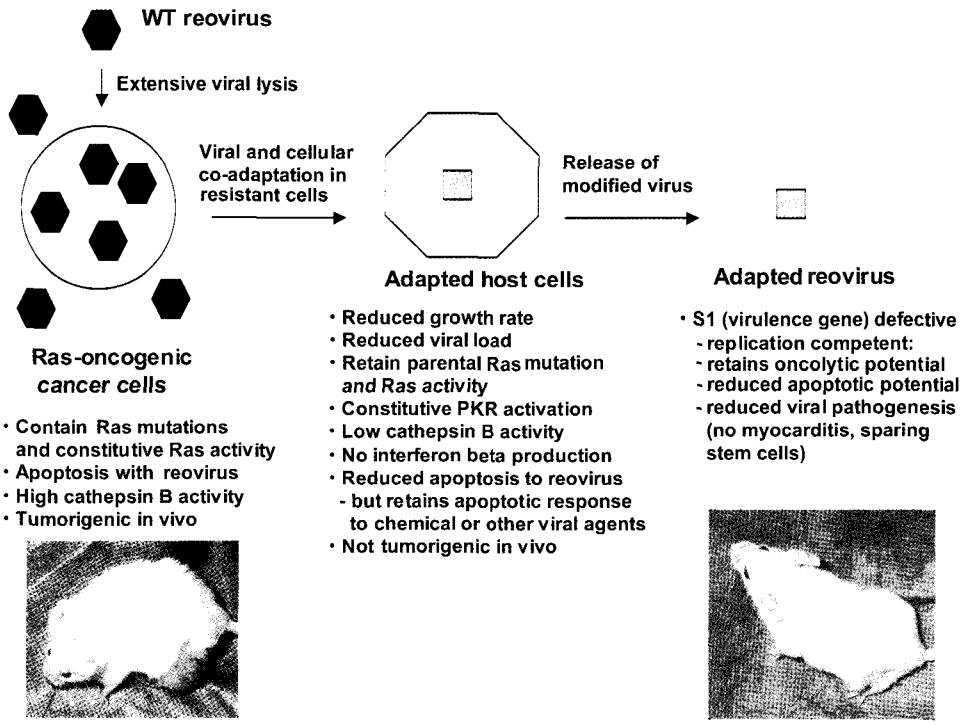

Fig. 2. Attenuation of reovirus by viral and cellular adaptation during persistent reovirus infection. Co-adaptation of reovirus and its cellular host resulted in mutation and truncation of the viral S1 gene coding sequence. This in turn caused an attenuation of viral apoptotic potential in healthy cells and tissues (Kim *et al.*, 2007a) while retaining the ability to eliminate tumors *in vivo*. The mouse on the left was injected in the flank with tumor cells showing massive proliferation, whereas the mouse on the right was injected with tumor cells plus attenuated virus, completely eliminating tumor growth.

lymphomas shows oncolytic potential in xenograft experiments (Alain *et al.*, 2006). Taken together, we conclude that genetically modified reoviruses derived from persistently infected cultured cells can still retain their innate oncolytic potential, and in some cases this is associated with a desirable reduction in damage to healthy tissue.

Conclusions and future directions

Many viruses have been shown to specifically target cancer cells while sparing normal counterparts, which ultimately led to the use of these viruses in clinical trials as potent anticancer agents (Ring, 2002; Norman and Lee, 2005; Roberts *et al.*, 2006). There are two dominant models to explain how different viruses may selectively target cancer cells while preserving normal cells:

A. Preferential viral tropism toward abnormal cellular signaling found in cancer cells

Cellular transformation is a multi-step process involving in most cases the accumulation of activated oncogenes and inactivated tumor suppressors via a series of point mutational events, chromosomal rearrangements and gene deletions or amplification. Interestingly, many oncolytic viruses appear to exploit abnormal cellular signaling pathways found in cancers in order to actively replicate and lyse them. For example and as mentioned above, reovirus can infect and kill cancer cells contain oncogenic Ras or Ras-dependent signaling pathways (Coffey *et al.*, 1998; Normal and Lee, 2005). Ras pathway signaling is activated in 30% or more of all cancer cases in humans, and this activation significantly contributes to tumor progression (Takai *et al.*, 2001; Duursma and Agami, 2003). Reovirus therefore has great potential to target Ras-oncogenic cancers and has currently progressed through a series of Phase I and II clinical trials with encouraging results (Stoeckel and Hay, 2006). Another example is myxoma virus, which is a rabbit-specific poxvirus pathogen that is being developed as an oncolytic agent because it is nonpathogenic in humans but nevertheless can infect and kill a wide spectrum of human cancer cells (Lun *et al.*, 2005; McFadden, 2005). Myxoma virus tropism at the cellular level is largely regulated by intracellular events downstream of virus binding and entry, rather than at the level of specific host receptors as is the case for many other viruses (McFadden, 2005). In this case, the pattern of selective oncolysis by myxoma virus suggests that mutational activation of Akt signalling pathways, frequently observed in many tumor types, leads to enhanced myxoma virus infection and oncolysis (Wang *et al.*, 2006). Alternatively, adenovirus variants have been used to target tumor suppressor (p53, Rb) defective cancer cells (Bischoff *et al.*, 1996; Heise *et al.*, 2000). The p53 gene is mutated or lost in ~50% of all human cancer cases (Carroll *et al.*, 1999; Morris, 2002), and thus adenovirus variants may be a powerful oncolytic agent targeting these tumors, especially when used in combination with other therapeutic strategies or with specific genetic modifications to enhance overall efficiency (Kim *et al.*, 2006). In some cases, we can even contemplate the possibility of combination therapy using multiple oncolytic viruses. For example, we found that fibrosarcoma cells that were initially susceptible to reovirus could occasionally acquire resistance to reoviral oncolysis (Kim *et al.*, 2007a) but even so retained their susceptibility to oncolytic adenovirus or other chemotoxic agents. Taken together, oncolytic viruses so far are mainly observed to utilize abnormally-regulated cellular signaling as observed in many cancers and thereby to exert selective viral oncolysis while largely sparing normal cells, with some exceptions as noted above.

B. Preferential viral tropism toward abnormal interferon or immune signalling found in cancer cells

Oncolytic viruses often exert a preferential viral tropism toward abnormal interferon signaling found in cancer cells. For instance, vesicular stomatitis virus (VSV) is a rhabdovirus, consisting of 5 genes encoded by a negative sense, single-stranded RNA genome. In nature, VSV infects insects as well as livestock, where it causes a relatively localized and non-fatal illness. The low pathogenicity of this virus is due in large part to its sensitivity to interferons, a class of proteins that are released into the tissues and bloodstream during infection. These molecules activate anti-viral defence programs that protect cells from infection and prevent spread of the virus. However, it has been shown that defects in these pathways can render cancer cells unresponsive to the protective effects of interferons and therefore highly sensitive to infection with VSV (Stojdl *et al.*, 2000). Since VSV undergoes a rapid cytolytic replication cycle, infection leads to death of the malignant cell and roughly a 1000-fold amplification of virus within 24 h. VSV is therefore highly suitable for therapeutic application, and several groups (Stojdl *et al.*, 2003; Ahmed *et al.*, 2004; Ebert *et al.*, 2005) have shown that systemically administered VSV can be delivered to a tumor site, where it replicates and induces disease regression, often leading to durable cures. Attenuation of the virus by engineering a deletion of Met-51 of the matrix protein blocks virtually all infection of normal tissues, while replication in tumor cells is unaffected (Stojdl *et al.*, 2003). In addition to VSV, measles virus and others have also been used to demonstrate that abnormal inferferon signalling as found in cancer cells can play an important role in viral oncolysis (Nakamura and Russell, 2004).

Summary

Modulation of viral pathogenicity is a critical factor for anticancer therapy, with the overall goal of minimizing damage to healthy tissue and organs while retaining efficient oncolytic potential, and in some cases this may include the targeting of viruses to specific forms of cancer, coupled with a well-defined spectrum of signalling abnormalities that confer viral susceptibility. Viral modulation or engineering can be induced by targeted mutation or deletion of specific virulence genes (Kirn, 2001; Kim *et al.*, 2007c) or by selection of variant viruses by multiple methods. For instance, vaccinia virus, which has been used as a vaccine to eradicate smallpox, subsequently has turned out to be an effective oncolytic virus (Thorne *et al.*, 2005). Moreover, naturally attenuated vaccine strains of measles virus can exert a powerful oncolytic effect against various types of cancers (Heinzerling *et al.*, 2005), and an attenuated vesicular stomatitis strain also exerts a powerful oncolytic effect (Stojdl *et al.*, 2003).

Can we envision a scenario whereby individual viral therapeutics are selected for use against specific forms of cancer?

Certainly it is already obvious that not all cancers respond equally well to the diverse viruses, and it will be important to better understand the pathways that govern the specific oncolytic responses, as well as those that are shared among diverse viral families. Intriguingly, one may speculate that certain tissues or cancer types should be especially susceptible to specific viral therapies. For example, reovirus normally replicates in tissues of the respiratory and enteric systems, and this natural tissue tropism may lead to the prediction that lung, gastric, or other enteric tumors may be especially susceptible to reoviral proliferation and oncolysis. The evidence at present is incomplete, but recent work suggests at least in animal models that azoxymethane-induced colon cancers respond favorably to this therapy (Alain *et al.*, 2007), thereby encouraging further exploration of this possibility.

These diverse oncolytic viruses, including various strains of natural or modified adenovirus, vaccinia virus, myxoma virus, measles virus, herpes simplex virus, vesicular stomatitis virus, reovirus and others preferentially infect and kill cancer cells due to enhanced viral tropism toward abnormally regulated cellular functions that are primarily manifest in diverse cancer cell types. Importantly, the various selected or modified viruses show minimal damage to healthy cells or tissues in the host while retaining their oncolytic potential. Thus we may envision in the near future a scenario where optimization of viral targeting to various human cancers, coupled with the use of oncolytic viruses in combination with other therapeutic strategies, and finally with the more accurate molecular characterization of individual tumor properties so that therapeutic approaches are offered to patients who will benefit most, together will provide increasingly effective strategies for treating this challenging family of diseases.

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References

Ahmed, M., S.D. Cramer, and D.S. Lyles. 2004. Sensitivity of prostate tumors to wild type and M protein mutant vesicular stomatitis viruses. *Virology* 330, 34-49.

Ahmed, R., W.M. Canning, R.S. Kauffman, A.H. Sharpe, J.V. Hallum, and B.N. Fields. 1981. Role of the host cell in persistent viral infection: coevolution of L cells and reovoirus during persistent infection. *Cell* 25, 325-332.

Alain, T., J.F. Wong, S. Urbanski, P. Lee, A.E. Kossakowska, R.N. Johnston, and P.L. Beck. 2007. Reovirus decreases azoxymethane-induced aberrant crypt foci and colon cancer in a rodent model. *Cancer Gene Ther.* (in press).

Alain, T., M. Kim, R.N. Johnston, S.J. Urbanski, A.E. Kossakowska, P.A.J. Forsyth, and P.W.K. Lee. 2006. The oncolytic effect in vivo of reovirus on tumour cells that have survived reovirus cell killing *in vitro*. *Br. J. Cancer* 95, 1020-1027.

Baer, G.S, D.H. Ebert, C.J. Chung, A.H. Erickson, and T.S. Dermody. 1999. Mutant cells selected during persistent reovirus infection do not express mature cathepsin L and do not support reovirus disassembly. *J. Virol.* 73, 9532-9543.

Baty, C.J. and B. Sherry. 1993. Cytopathogenic effect in cardiac myocytes but not in cardiac fibroblasts is correlated with reovirus-induced acute myocarditis. *J. Virol.* 67, 6295-6298.

Bischoff, J.R., D.H. Kirn, A.Williams, C. Heise, S. Horn, M. Muna, L. Ng, J.A. Nye, A. Sampson-Johannes, A. Fattaey, and F. McCormick. 1996. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 274, 373-376.

Bos, J.L. 1989. Ras oncogenes in human cancer: a review. *Cancer Res.* 49, 4682-4689.

Carroll, P.E, M. Okuda, H.F. Horn, P. Biddinger, P.J. Stambrook, L.L. Gleich, Y.Q. Li, P. Tarapore, and K. Fukasawa. 1999. Centrosome hyperamplification in human cancer: chromosome instability induced by p53 mutation and/or Mdm2 overexpression. *Oncogene* 18, 1935-1944.

Coffey, M.C., J.E. Strong, P.A. Forsyth, and P.W. Lee. 1998. Reovirus therapy of tumors with activated Ras pathway *Science* 282, 1332-1334.

DeBiasi, R.L., B.A. Robinson, B. Sherry, R. Bouchard, R.D. Brown, M. Rizeq, C. Long, and K.L. Tyler. 2004. Caspase inhibition protects against reovirus-induced myocardial injury *in vitro* and *in vivo*. *J. Virol.* 78, 11040-11050.

Dermody, T.S. 1998. Molecular mechanisms of persistent infection by reovirus. *Curr. Top Microbiol. Immunol.* 233(Pt 2), 1-22.

Dichter, M.A. and H.L. Weiner. 1984. Infection of neuronal cell cultures with reovirus mimics *in vitro* patterns of neurotropism. *Ann. Neurol.* 16, 603-610.

Duncan, M.R., S.M. Stanish, and D.C. Cox. 1978. Differential sensitivity of normal and transformed human cells to reovirus infection. *J. Virol.* 28, 444-449.

Duursma, A.M. and R. Agami. 2003. Ras interference as cancer therapy. *Semin. Cancer Biol.* 13, 267-273.

Ebert, D.H, S.A. Kopecky-Bromberg, and T.S. Dermody. 2004. Cathepsin B is inhibited in mutant cells selected during persistent reovirus infection. *J. Biol. Chem.* 279, 3837-3851.

Ebert, O., S. Harbaran, K. Shinozaki, and S.L. Woo. 2005. Systemic therapy of experimental breast cancer metastases by mutant vesicular stomatitis virus in immune-competent mice. *Cancer Gene Ther.* 12, 350-358.

Egan, C., M. Kim, and R.N. Johnston. 2003. Increased expression of human c-Myc renders cells sensitive to reovirus oncolysis. AACR Annual General Meeting, Washington, D.C., USA.

Haller, B.L, M.L. Barkon, G.P. Vogler, and H.W. Virgin 4th. 1995. Genetic mapping of reovirus virulence and organ tropism in severe combined immunodeficient mice: organ-specific virulence genes. *J. Virol.* 69, 357-364.

Hashiro, G., P.C. Loh, and J.T. Yau. 1977. The preferential cytotoxicity of reovirus for certain transformed cell lines. *Arch. Virol.* 54, 307-315.

Heggie, A.D. and L. Gaddis. 1979. Effects of viral exposure of the two-cell mouse embryo on cleavage and blastocyst formation *in vitro*. *Pediatr. Res.* 13, 937-941.

Heinzerling, L., V. Kunzi, P.A. Oberholzer, T. Kundig, H. Naim, and R. Dummer. 2005. Oncolytic measles virus in cutaneous T-cell lymphomas mounts antitumor immune responses *in vivo* and targets interferon-resistant tumor cells. *Blood* 106, 2287-2294.

Heise, C., T. Hermiston, L. Johnson, G. Brooks, A. Sampson-Johannes, A. Williams, L. Hawkins, and D. Kirn. 2000. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nat. Med.* 6, 1134-1139.

Jackson, G.G. and R.L. Muldoon. 1973. Viruses causing common respiratory infection in man. IV. Reoviruses and adenoviruses. *J. Infect. Dis.* 128, 811-833.

Jun, H.S. and J.W. Yoon. 2003. A new look at viruses in type 1

diabetes. *Diabetes Metab. Res. Rev.* 19, 8-31.

Kim, J.H., Y.S. Lee, H. Kim, J.H. Huang, A.R. Yoon, and C.O. Yun. 2006. Relaxin expression from tumor-targeting adenoviruses and its intratumoral spread, apoptosis induction and efficacy. *J. Natl. Cancer Inst.* 98, 1482-1493.

Kim, M. 2005. Mechanisms of resistance to reoviral oncolysis. Ph. D. Thesis. University of Calgary, Alberta, Canada.

Kim, M., C. Egan, T. Alain, S.J. Urbanski, P.W.K. Lee, P.A.J. Forsyth, and R.N. Johnston. 2007a. Acquired resistance to reoviral oncolysis in Ras-transformed fibrosarcoma cells. *Oncogene* 26, 4124-4134.

Kim, M., N. Nieden, S.D. Loken, S.J. Urbanski, P.W.K. Lee, D.E. Rancourt, and R.N. Johnston. 2007b. Safety of attenuated reovirus on the developmental potential of embryonic stem cells. The 4th International Conference on Oncolytic Viruses As Cancer Therapeutics. Scottsdale, AZ, USA.

Kim, M., T. Alain, S.J. Urbanski, A.E. Kossakowska, P.W.K. Lee, P.A.J. Forsyth, and R.N. Johnston. 2007c. An attenuated reovirus isolated from persistent reovirus infection exerts viral oncolysis with reduced pathogenicity. The 4th International Conference on Oncolytic Viruses As Cancer Therapeutics. Scottsdale, AZ, USA.

Kirn, D. 2001. Oncolytic virotherapy for cancer with the adenovirus dl1520 (Onyx-015): results of phase I and II trials. *Expert Opin. Biol. Ther.* 1, 525-538.

Loken, S.D., K. Norman, K. Hirasawa, M. Nodwell, W.M. Lester, and D.J. Demetrick. 2004. Morbidity in immunosuppressed (SCID/NOD) mice treated with reovirus (dearing 3) as an anti-cancer biotherapeutic. *Cancer Biol. Ther.* 3, 734-738.

Lun, X., W. Yang, T. Alain, Z.Q. Shi, H. Muzik, J.W. Barrett, G. McFadden, J. Bell, M.G. Hamilton, D.L. Senger, and P.A. Forsyth. 2005. Myxoma virus is a novel oncolytic virus with significant antitumor activity against experimental human gliomas. *Cancer Res.* 65, 9982-9990.

Mann, M.A, K.L. Tyler, D.M Knipe, and B.N. Fields. 2002. Type 3 reovirus neuroinvasion after intramuscular inoculation: viral genetic determinants of lethality and spinal cord infection. *Virology* 303, 213-221.

McFadden, G. 2005. Poxvirus tropism. *Nat. Rev. Microbiol.* 3, 201-213.

Minuk, G.Y., N. Rascanin, R.W. Paul, P.W. Lee, K. Buchan, and J.K. Kelly. 1987. Reovirus type 3 infection in patients with primary biliary cirrhosis and primary sclerosing cholangitis. *J. Hepatol.* 5, 8-13.

Minuk, G.Y., R.W. Paul, and P.W. Lee. 1985. The prevalence of antibodies to reovirus type 3 in adults with idiopathic cholestatic liver disease. *J. Med. Virol.* 16, 55-60.

Morris, S.M. 2002. A role for p53 in the frequency and mechanism of mutation. *Mutat. Res.* 511, 45-62.

Nakamura, T. and S.J. Russell. 2004. Oncolytic measles viruses for cancer therapy. *Expert Opin. Biol. Ther.* 4, 1685-1692.

Nibert, M.L. and L.A. Schiff. 2001. Reoviruses and their replication. p. 1679-1728, *In* B.N. Fields, D.M Knipe, and P.M. Howley, (eds.). Fields Virology, Lippincott-Raven Publisher, Philadelphia, USA.

Norman, K.L. and P.W. Lee. 2005. Not all viruses are bad guys: the case for reovirus in cancer therapy. *Drug Discov. Today* 10, 847-855.

Priscott, P.K. 1983. The growth of reovirus 3 in cultured rat embryos and implications for human reproductive failure. *Br. J. Exp. Pathol.* 64, 467-473.

Richardson, S.C., R.F. Bishop, and A.L. Smith. 1994. Reovirus serotype 3 infection in infants with extrahepatic biliary atresia or neonatal hepatitis. *J. Gastroenterol. Hepatol.* 9, 264-268.

Ring, C.J. Cytolytic viruses as potential anti-cancer agents. 2002. *J. Gen. Virol.* 83, 491-502.

Rosen, L., H.E. Evans, and A. Spickard. 1963. Reovirus infections in human volunteers. *Am. J. Epidemiol.* 77, 29-37.

Roberts, M.S., R.M. Lorence, W.S. Groene, and M.K. Bamat. 2006. Naturally oncolytic viruses. *Curr. Opin. Mol. Ther.* 8, 314-321.

Russell, S.J. 2002. RNA viruses as virotherapy agents. *Cancer Gene Ther.* 9, 961-966.

Sabin, A.B. 1959. Reoviruses. A new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. *Science* 130, 1387-1389.

Steele, T.A. and C.C Hauser. 2005. The role of interferon-alpha in a successful murine tumor therapy. *Exp. Biol. Med.* 230, 487-493.

Steele, T.A. and D.C. Cox. 1995. Reovirus type 3 chemoimmunotherapy of murine lymphoma is abrogated by cyclosporine. *Cancer Biother.* 10, 307-315.

Stoeckel, J. and J.G. Hay. 2006. Drug evaluation: Reolysin-wild-type reovirus as a cancer therapeutic. *Curr. Opin. Mol. Ther.* 8, 249-260.

Stojdl, D.F., B. Lichty, S. Knowles, R. Marius, H. Atkins, N. Sonenberg, and J.C. Bell. 2000. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat. Med.* 6, 821-825.

Stojdl, D.F., B.D. Lichty, B.R. TenOever, J.M. Paterson, A.T. Power, S. Knowles, R. Marius, J. Reynerd, L. Poliquin, H. Atkins, E.G. Brown, R.K. Durbin, J.E. Durbin, J. Hiscott, and J.C. Bell. 2003. VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. *Cancer Cell.* 4, 263-275.

Strong, J.E., M.C. Coffey, D. Tang, P. Sabinin, and P.W. Lee. 1998. The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. *EMBO J.* 17, 3351-3362.

Strong, J.E. and P.W. Lee. 1996. The v-erbB oncogene confers enhanced cellular susceptibility to reovirus infection. *J. Virol.* 70, 612-616.

Takai, Y., T. Sasaki, and T. Matozaki. 2001. Small GTP-binding proteins. *Physiol. Rev.* 81, 153-208.

Terheggen, F., E. Benedikz, P.H. Frissen, and K. Brinkman. 2003. Myocarditis associated with reovirus infection. *Eur. J. Clin. Microbiol. Infect. Dis.* 22, 197-198.

Thorne, S.H., D.L. Bartlett, and D.H. Kirn. 2005. The use of oncolytic Vaccinia viruses in the treatment of cancer: a new role for an old ally? *Curr. Gene Ther.* 5, 429-443.

Tyler, K.L. 2001. Mammalian reoviruses, p. 1729-1745. *In* B.N. Fields, D.M. Knipe, and P.M. Howley (eds), Fields Virology, Lippincott-Raven, Philadelphia, USA.

Wang, G., J.W. Barrett, M. Stanford, S.J. Werden, J.B. Johnston, X. Gao, M. Sun, J.Q. Cheng, and G. McFadden. 2006. Infection of human cancer cells with myxoma virus requires Akt activation via interaction with a viral ankyrin-repeat host range factor. *Proc. Natl. Acad. Sci. USA* 103, 4640-4645.

Weiner, H.L., D. Drayna, D.R. Averill, Jr., and B.N. Fields. 1977. Molecular basis of reovirus virulence: role of the S1 gene. *Proc. Natl. Acad. Sci. USA* 74, 5744-5748.

Weiner, H.L., M.L. Powers, and B.N. Fields. 1980. Absolute linkage of virulence and central nervous system cell tropism of reoviruses to viral hemagglutinin. *J. Infect. Dis.* 141, 609-616.