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REVIEW ARTICLE

Cytolytic viruses as potential anti-cancer agents

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The resistance of cancers to conventional therapies has inspired the search for novel strategies. One such approach, namely gene therapy, is based upon the introduction of genes such as those encoding suicide proteins, tumour suppressor proteins or cytokines into tumour cells by means of a genetic vector. The efficiency with which viruses transfer their genes from one host cell to another has led to the widespread use of viruses as genetic vectors. For safety reasons, such virus vectors are generally replication-defective but, unfortunately, this has limited the efficacy of treatment by restricting the number of cells to which the therapeutic gene is delivered. For this reason, the use of replication-competent viruses has been proposed, since virus replication would be expected to lead to amplification and spread of the therapeutic genes *in vivo*. The replication of many viruses results in lysis of the host cells. This inherent cytotoxicity, together with the efficiency with which viruses can spread from one cell to another, has inspired the notion that replication-competent viruses could be exploited for cancer treatment. Some viruses have been shown to replicate more efficiently in transformed cells but it is unlikely that such examples will exhibit a high enough degree of tumour selectivity, and hence safety, for the treatment of patients. Our increasing knowledge of the pathogenesis of virus disease and the ability to manipulate specific regions of viral genomes have allowed the construction of viruses that are attenuated in normal cells but retain their ability to lyse tumour cells. Such manipulations have included modifying the ability of viruses to bind to, or replicate in, particular cell types, while others have involved the construction of replication-competent viruses encoding suicide proteins or cytokines. Naturally occurring or genetically engineered oncolytic viruses based upon adenovirus, herpes simplex virus, Newcastle disease virus, poliovirus, vesicular stomatitis virus, measles virus and reovirus have been described. The results of animal studies are encouraging and a number of viruses are now being evaluated in clinical trials.

Introduction

The study of viruses has led to a number of major breakthroughs in our understanding of the basic principles of biology (reviewed by Levine, 1996), including cell cycle control and carcinogenesis. Great insights into the mechanisms of cell growth regulation have been gained, not only from the study of the non-cytolytic murine and avian retroviruses, but also DNA viruses that usually cause cell lysis but when infecting cells from unnatural hosts, undergo an abortive non-cytolytic infection (Nevins & Vogt, 1996; Weiss, 1998). The limited efficacy and toxicity of current cancer therapies have been the impetus for the search for novel approaches to treatment. One such approach is gene therapy, which seeks to treat cancer by the introduction of genes that will result in destruction of the tumour from within, or will enhance an immune response against it. Viruses are frequently chosen as vehicles for such genes because they have evolved very efficient mechanisms of gene transfer (transduction) and expression (Ring & Blair, 2000). Most of the cancer gene therapy trials to date utilize replication-defective viruses for gene transduction (reviewed by Walther & Stein, 2000). Such viruses have generally been genetically crippled so that the virus replication cycle is aborted once the virus has entered the cell. The viral genome, however, is still able to express the gene of interest. The use of replication-defective viruses is of

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particular importance where diseases other than cancer are being treated, in which the survival of the transduced cell is a necessity. If, however, one is attempting to transduce tumour cells and destroy them then one may take advantage of any cytotoxic effects a virus may exhibit. In very simplistic terms, viruses cause disease either directly, by affecting the physiology of the cells they infect, or indirectly, by the host organism mounting an attack upon the virus and virus-infected cells in an attempt to remove the pathogen. The most dramatic effect that viruses have on their host cells is lysis. Since some viruses can directly damage cells, one may be able to exploit these pathogens to mediate lysis of specific cell populations such as tumours. Virus-mediated destruction of tumours has been the subject of recent reviews (Heise & Kirn, 2000; Martuza, 2000; Kirn *et al*., 2001); however, this is a fast-moving field and there have many significant developments in the last few months to a year. This review describes the ways in which viruses have been manipulated in order to target and enhance their cytolytic properties with a view to their exploitation as anti-cancer agents.

Infection of animal cells with viruses often stimulates the cellular self-destruct mechanism (a process known as apoptosis), presumably in an attempt to limit infection (Everett & McFadden, 1999). In the case of adenoviruses, upon completion of the virus replication cycle, the viral 'death protein' mediates cell lysis, allowing release of the progeny virions (Tollefson *et al*., 1996). Many bacteriophages also encode specific cell lysis proteins which act to facilitate the exit of progeny virions from host bacterial cells. In general, however, viruses that infect animal cells bring about cell destruction as a result of the heavy biochemical demands that the virus makes upon its host cell. For viruses to subvert the cellular biochemistry in order to replicate, there is often inhibition of vital processes such as host gene transcription, protein translation, intracellular transport and other effects such as membranous vesicle accumulation, increased plasma membrane permeability, fusion of cell membranes and depolymerization of the cytoskeleton (Flint *et al*., 2000).

The idea of using viruses in the treatment of cancer is not new. Observations made in the early 1920s indicated that viruses replicated in and lysed murine and other experimental tumours. Amongst the earliest reports on regression of human tumours is the case of cervical carcinoma that regressed after inoculation of the patient with attenuated rabies vaccine (De Pace, 1912). In addition, there are reports of remissions of Burkitt's and Hodgkin's lymphomas following natural infections with measles virus (Bluming & Ziegler, 1971; Taqi *et al*., 1981). If indeed the viruses did contribute to tumour regression in these cases, it is possible that the effect was mediated by the virus stimulating an anti-tumour immune response rather than infecting and destroying the tumour directly. Intentional inoculations of live viruses into tumour patients were initiated in the late 1940s; however, these very seldom resulted in complete remissions (reviewed by Sinkovics & Horvath, 1993).

Naturally occurring oncotropic viruses

There are a number of examples of naturally occurring viruses that are tumour-selective in their replication and cytolysis. Autonomously replicating parvoviruses such as minute virus of mice and H1, human reovirus and vesicular stomatitis virus (VSV) have been shown to replicate more efficiently in transformed cell lines relative to non-transformed cell lines (Hashiro *et al*., 1977; Rommelaere & Tattersall, 1990; Stojdl *et al*., 2000). Furthermore, human reovirus appears to exhibit tumour specificity *in vivo*. Using an intracranial mouse model of human glioma, inoculation of cranial tumours with reovirus has led to significant prolongation in the survival of mice compared to those treated with inactivated reovirus (Wilcox *et al*., 2001). The precise mechanisms for tumour selectivity are poorly understood and are likely to differ with each virus. Unlike other DNA viruses, the autonomous parvoviruses are unable to force resting cells into S phase and are therefore incapable of undergoing genome replication in quiescent cells (Rommelaere & Tattersall, 1990). Therefore, the efficiency with which parvoviruses replicate in transformed cell lines relative to normal cells is probably due to the aberrant cell cycle control exhibited by transformed cells. Reovirus and VSV are RNA viruses and encode their own polymerase proteins for replicating their genomes. As a result, these viruses are not as dependent upon S phase as some DNA viruses are. Strong *et al*. (1998) showed that activation of the *ras* pathway relieved a block on reovirus gene translation, offering at least a partial explanation for the apparent selectivity for transformed cells. Stojdl *et al*. (2000) has recently shown that VSV, which is exquisitely sensitive to interferon, exploits defects in the interferon pathway of transformed cells, enabling its replication in such cells. Some naturally occurring viruses of veterinary importance have been adapted by serial passage in tumour cells to increase their oncolytic efficacy (Hammon *et al*., 1963; Yohn *et al*., 1968). Newcastle disease virus (NDV) strains adapted to Ehrlich ascites carcinoma cells (Cassel *et al*., 1983) or human melanoma cells (Ahlert & Schirrmacher, 1990) are two examples. NDV was first reported as having oncolytic activity in the mid-1950s (Flanagan *et al*., 1955) and lysates, prepared from NDV-infected tumour explants (known as 'viral oncolysates'), have been administered to cancer patients since the mid-1960s in attempts to augment the anti-tumour immune response (reviewed by Sinkovics & Horvath, 2000). Recent studies into the oncolytic activity of NDV have yielded encouraging results. Phuangsab *et al*. (2001), using murine xenograft models, demonstrated significant inhibition of tumour growth following intratumoural administration of NDV. Furthermore, complete tumour regression was observed in nine of 12 mice bearing neuroblastoma tumour xenografts after a single intraperitoneal injection of NDV. These results are likely to inspire further investigations into the potential use of NDV as an anti-cancer agent.

Engineering tumour selectivity into viruses

Transformed cells exhibit biochemical and functional differences from their normal counterparts. For example, transformed cells exhibit different patterns of gene expression and often display different proteins on their surfaces. Currently, attempts are being made to exploit these differences with a view to generating tumour-specific viruses. In general terms, virus replication can be subdivided into the attachment of virions to the host cell, internalization, uncoating, transcription of the viral genome, translation of the viral RNA and the assembly and release of progeny virions (Ring & Blair, 2000). Each of these processes could represent opportunities for achieving tumour specificity. For example, tumour selectivity may be achieved by modifying viral attachment proteins such that the resulting viruses bind selectively to a tumour-specific cell surface molecule (Lindblom & Liljegren, 2000). Furthermore, tumour selectivity may be achieved by linking viral protein coding regions to the promoters or enhancers of cellular genes that are upregulated in, or expressed solely in, tumour cells (Nettelbeck *et al.*, 2000).

The selection of the optimal treatment for a cancer patient depends upon the type and location of the tumour and this is also likely to be the case with therapies based upon cytolytic viruses. Brain tumours such as glioblastoma represent masses of proliferating cells within non-proliferating normal brain tissue. In this case, a neurotropic virus that can only replicate in cycling cells may offer the necessary level of tumour specificity. Some mutants of the neurotropic herpes simplex virus (HSV) can only replicate in proliferating cells and thus have potential as therapeutic agents for brain tumours. Deletion of genes encoding proteins that are involved in nucleic acid metabolism, such as thymidine kinase and ribonucleotide reductase, can generate a virus that is incapable of replicating in resting cells. Moreover, deletion of the genes encoding thymidine kinase and infected cell protein 6 (ICP6; the large subunit of ribonucleotide reductase) generates viruses with a selective ability to destroy gliomas in immunocompetent rats (Jia *et al.*, 1994; Martuza *et al.*, 1991; Boviatsis *et al.*, 1994; Mineta *et al.*, 1994; Marbert *et al.*, 1993). Deletion of both copies of the gene encoding ICP34.5 also generates mutants that are unable to replicate in the brain or to cause encephalitis. These mutants are, however, able to replicate in brain tumour tissue (Bolovan *et al.*, 1994; Chou *et al.*, 1990; MacLean *et al.*, 1991). Interestingly ICP34.5 is not involved in nucleic acid metabolism but in counteracting the interferon-inducible PKR-mediated block on virus replication (Chou *et al.*, 1990). These recombinant viruses are avirulent upon intracerebral inoculation in normal mice but have been shown to slow tumour growth and prolong the survival of nude mice implanted with human glioma cells by direct cytolysis. Recombinant HSVs bearing multiple mutations in HSV genes have also been evaluated (Mineta *et al.*, 1995; Kramm *et al.*, 1997; Pyles *et al.*, 1997).

Selective lysis of glioma cells by HSV mutants occurs because the tumour cells are proliferating, whereas the surrounding normal cells are quiescent. As such, the cytolysis is dependent upon cellular proliferation rather than any specific difference between malignant and normal cells. To achieve specific lysis of tumour cells at other anatomical sites where surrounding normal cells may also be proliferating, a further level of tumour specificity has to be sought. As stated earlier, this may be achieved either by restricting the binding to, or expression of the virus in, tumour cells.

Attempts to achieve tumour-selective binding and internalization of virus

The manner in which tumour-selective binding and internalization is achieved is dependent upon the virus and its natural tropism. For example, with a virus that naturally infects only a very narrow range of cell types, one may just have to introduce a new binding specificity into the virion. However, for a virus that naturally infects numerous cell types, not only may a new binding specificity have to be introduced, but the natural binding interaction will have to be abolished. To date, this has proved to be particularly challenging.

The initial steps of adenovirus infection involve two sequential virus–cell interactions, each being mediated by a specific protein component of the viral capsid. The primary binding of the virus to the cell surface coxsackie and adenovirus receptor (CAR) is mediated by the knob domain of the fibre protein. This is followed by internalization of the virion within endosomes. The virions escape from endosomes by triggering its acidification via a secondary interaction of the arginine-glycine-aspartic acid (RGD) motif of the viral penton base protein with the cellular integrins $\alpha v\beta 3$ and $\alpha v\beta 5$. Following escape, the partially dismantled virus translocates to the nuclear pore complex and releases its genome into the nucleoplasm, where the subsequent steps of virus replication take place (Nemerow, 2000). The utility of adenovirus vectors is limited due to the low efficiency of infection of cells expressing low levels of CAR. Adenovirus types 2 and 5, upon which most of the adenovirus vectors constructed to date are based (Ring, 1996), replicate in a wide variety of cell types; however, previous studies have demonstrated a low level of virus binding to smooth muscle, endothelium, haematopoietic stem cells, macrophages and T cells. Substitution of the natural fibre protein by those of other adenovirus types has increased the infectivity of adenovirus type 5 vectors for these cell types (Shayakhmetov *et al.*, 2000; Havenga *et al.*, 2001). Alternative approaches to extend the tropism of adenovirus to specific cell types have involved the genetic modification of the fibre protein gene. Wickham *et al.* (1997) modified the C terminus of the adenoviral fibre protein either by the addition of an RGD-containing peptide or the addition of seven lysine residues. The addition of RGD was shown to enhance transduction of endothelial and smooth

muscle cells expressing αv integrins. Furthermore, the addition of seven lysines enhanced the transduction of cells lacking high levels of CAR, including macrophages, endothelium, smooth muscle, fibroblasts and T cells. A second group has reported modifying the fibre protein by the addition of lysine residues. Shinoura *et al*. (1999) constructed an adenovirus bearing a fibre with a stretch of 20 lysine residues added to its C terminus and demonstrated this virus to infect glioma cells with greater efficiency than a virus bearing an unmodified fibre protein. Furthermore, using a glioma xenograft mouse model, the anti-tumour effect of the fibre-modified virus was significantly stronger than the unmodified virus, suggesting that this fibre modification may be a promising approach for treating glioma. The precise mechanism for the enhanced infection by the lysine-modified fibre mutant is unclear, however. Dmitriev *et al*. (1998) have also shown that the incorporation of an RGD-containing peptide in the H1 loop of the fibre knob domain results in the ability of the virus to utilize an alternative receptor during the cell entry process. They also showed that due to the expanded tissue tropism, the virus was capable of infecting primary tumour cells and tumour cell lines more efficiently than unmodified virus (Dmitriev *et al*., 1998; Kasono *et al*., 1999). The RGD/fibre modification was subsequently introduced into a virus bearing an E1A mutation that abolishes binding of E1A to pRB, and showed that the fibre-modified virus ('Ad5-Δ24RGD') replicated in and lysed tumour cells *in vitro* more efficiently that the virus that possessed a wild-type fibre protein ('Ad5-Δ24') (Suzuki *et al*., 2001).

Gu *et al*. (1999) have successfully redirected cell binding and uptake of an adenovirus through fibroblast growth factor receptors (FGFRs). Replication-defective adenovirus vectors encoding either β-galactosidase (β-gal), green fluorescent protein (GFP) or HSV thymidine kinase (HSV-tk) were incubated with a virus-neutralizing, anti-fibre protein Fab antibody fragment conjugated to fibroblast growth factor 2 (FGF2). Conjugate-treated or untreated β-gal–virus was administered intravenously to mice to assess the degree of β-gal expression in liver. It was shown that there was up to 20-fold less β-gal activity in the livers of mice infected with the FGF-conjugated virus compared to untreated virus, suggesting that FGF-conjugated virus had a reduced liver tropism. To confirm whether infection of conjugated virus could occur through a CAR-independent mechanism, FGF-conjugated and control GFP–adenovirus was incubated with B16F0 murine melanoma cells. These cells express FGF receptors and have previously been shown to be resistant to adenovirus infection *in vitro*. Incubation with FGF-conjugated GFP–virus was shown to result in a significantly higher number of transduced cells, and higher levels of GFP expression, than when cells were incubated with unconjugated virus. A mouse model of metastatic melanoma was used to demonstrate whether conjugation of HSV-tk-expressing virus enhanced the efficiency of anti-tumour treatment. B16F0 melanoma cells were exposed to either FGF-conjugated HSV-tk virus or unconjugated HSV-tk virus and administered intraperitoneally into mice. Ganciclovir (GCV) treatment was then initiated 1 day after tumour cell inoculation. Mice whose tumour inocula had been treated with FGF-conjugated virus demonstrated a 260% increase in lifespan compared to those mice whose tumour inocula had been treated with unconjugated virus. More recently, Printz *et al*. (2000) have shown, using an intraperitoneal model of ovarian cancer, that FGF-conjugated adenovirus directed an increased level of transgene expression in tumour tissue compared to unconjugated virus. Both these studies suggest that redirecting the native tropism of adenovirus may offer therapeutic benefit. It should be noted, however, that in these cases, the virus was replication-defective and the virus had not been genetically modified. It remains to be seen if genetic modification of the fibre protein of a replication-competent virus results in selective lysis of FGFR-expressing cells.

Laquerre *et al*. (1998) have attempted to construct a targeted HSV vector. In order to do this, the natural tropism of HSV, mediated by the interaction of the viral glycoproteins B (gB) and C with cell surface glucosaminoglycans (Spear, 1993; Herold *et al*., 1994), had to be eliminated and a new ligand, capable of binding to cell surface receptor, incorporated into the virus envelope. A recombinant HSV was constructed that was deleted for gC and the heparan sulphate-binding domain of gB and engineered to encode a chimeric protein composed of N-terminally truncated gC and full-length erythropoietin (epo). The resulting virus was shown to have incorporated the gC/epo chimera molecule and to stimulate proliferation of the EPO-dependent FD-EPO cell line. These cells, however, were refractory to productive infection by the EPO-containing virus, indicating that the cells could bind the EPO-displaying virus, but that there was a subsequent block in the replication cycle.

Significant progress has been made in modifying the tropism of measles virus. This has been done by fusing the viral attachment protein, the haemagglutinin (H), either with single chain antibodies or growth factors. Schneider *et al*. (2000) constructed recombinant measles viruses displaying either epidermal growth factor (EGF) or insulin-like growth factor 1 (IGF1) on their H proteins. The H/EGF and H/IGF1 viruses were able to bind and replicate in rodent cells expressing the EGF receptor and IGF receptor, respectively. Since rodent cells lack the usual measles receptor, CD46 (Dorig *et al*., 1993; Naniche *et al*., 1993), this demonstrated that virus binding had been successfully redirected. Indeed, this was the first demonstration that large specificity domains covalently linked to a viral glycoprotein support not only binding to a new receptor but also efficient cell entry via the targeted receptor. Further investigations indicated that the viruses had maintained their ability to bind to cells via CD46, however. The same group constructed a recombinant measles virus that displayed a single-chain antibody specific for the tumour-associated carcinoembryonic antigen (CEA) on its haemag-

glutinin protein (Hammond *et al*., 2001). The H/CEA virus was able to infect CEA-expressing cells; however, like the H/EGF and H/IGF1 viruses, this virus also maintained its ability to bind to cells via CD46. The ability to extend the tropism of measles virus is of particular significance in the light of a recent report describing that measles virus can replicate in and destroy human lymphoma cells in a mouse xenograft model (Grote *et al*., 2001). If binding of measles virus to CD46 can be abolished and the viral tropism redirected to a tumour-specific receptor, the modified measles virus could form the basis of an anti-lymphoma therapy in patients.

There are numerous examples where the tropism of a virus can be altered by substituting its attachment protein with that of a second, unrelated virus. For example, the relatively restricted host cell range of murine type C retroviruses has been overcome by substituting the retroviral envelope glycoprotein (env) by that encoded by the rhabdovirus VSV (VSV-G) (Burns *et al*., 1993; Akkina *et al*., 1996). Conversely, a replication-competent VSV has been constructed by replacing the VSV-G gene with a hybrid gene encoding the extracellular and transmembrane domains of the envelope protein of a retrovirus, namely human immunodeficiency virus (HIV), fused to the cytoplasmic domain of VSV-G (Boritz *et al*., 1999). Infectivity of the recombinant VSV was blocked by antibodies to CD4 and CXCR4, indicating that binding of the virus had indeed been redirected to those cell surface molecules bound by HIV. Furthermore, substitution of the VSV-G protein gene by the genes encoding CD4 and CXCR4 has been shown to restrict virus replication to cells infected with HIV (Schnell *et al*., 1997). The observation that VSV replicates more efficiently in transformed cells (Stojdl *et al*., 2000) and the fact that it can be engineered to bind to specific cell receptors and encode foreign genes (Schnell *et al*., 1996; Johnson *et al*., 1997; Kahn *et al*., 1999; Rose *et al*., 2000) suggest that VSV could form the basis for a tumour-selective anti-cancer agent in the future.

Attempts to achieve tumour-selective virus replication and lysis

An alternative approach in an attempt to achieve tumour specificity is to exploit tumour-specific defects in the control of cell proliferation. DNA viruses such as adenovirus, papillomavirus and polyomaviruses encode proteins that force infected cells into S phase so that viral DNA can replicate efficiently. These viral proteins have been shown to bind and inactivate the cellular pRB and p53 proteins (reviewed by Nevins & Vogt, 1996). pRB has a role in controlling cell division and targeting of this protein by viral proteins allows cells to escape the normal checks on proliferation. The hypophosphorylated form of pRB, which is present in the G_0 and G_1 phases of the cell cycle, binds to a number of transcription factors, most notably the E2F family. Upon phosphorylation of pRB by cyclin-dependent kinases (CDKs), these transcription factors are released from pRB, allowing them to mediate transcriptional activation and progression through the G_1/S boundary. The adenoviral E1A, papillomavirus E7 and simian virus 40 large T proteins circumvent this negative regulation by binding to hypophosphorylated pRB and displacing E2F, allowing cells to progress into S phase in the absence of normal mitogenic signals (Nevins, 2001). The cellular p53 protein is also a negative regulator of cell growth. Unlike pRB, however, p53 is not a constitutive component of cell cycle control but is induced in response to DNA damage, mediating G_1 arrest and apoptosis (Bates & Vousden, 1996). p53 is a transcription factor that mediates G_1 arrest via induction of the $p21^{WAF1/CIP1}$ CDK inhibitor (Woods & Vousden, 2001). The adenoviral E1B-55K and the papillomavirus E6 proteins inhibit the binding of p53 to DNA and hence its transcriptional activating properties, in turn inhibiting the expression of the CDK inhibitor and allowing cell cycle progression. A mutant adenovirus known as ONYX-015 (originally termed *dl*1520) lacks the E1B-55K gene. ONYX-015 was originally reported to replicate only in tumours that lacked a functional p53 protein (Bischoff *et al*., 1996); however, subsequent investigations have suggested that replication of ONYX-015 may not be entirely dependent upon p53 status (Goodrum & Ornelles, 1998; Harada & Berk, 1999; Rothmann *et al*., 1998; Turnell *et al*., 1999). It now appears that differences in cell killing by ONYX-015 may also be due to differences in infectivity or cell permissiveness rather than just p53 status (Steegenga *et al*., 1999). The recent report that CAR is abundantly expressed in tumours, but is restricted to basal epithelial layers in non-tumour tissues (Hutchin *et al*., 2000), supports the differential infectivity theory. Phase I and II clinical trials of ONYX-015 have been encouraging (Kirn *et al*., 1998; Ganly *et al*., 2000; Nemunaitis *et al*., 2000) and intratumoural and peritumoural administration of ONYX-015 to patients with advanced head and neck cancer has shown that the virus replicates in tumour tissue but not in adjacent normal tissue. Moreover, significant tumour regressions ($> 50\%$ tumour volume) were observed in 21% of evaluable virus-treated patients (Nemunaitis *et al*., 2000). Combining ONYX-015 treatment with the administration of the cytotoxic drugs cisplatin and 5-fluorouracil has been shown to result in tumour regressions in 63% of evaluable patients (Khuri *et al*., 2000).

Adenoviruses designated KD1 and KD3 contain two small deletions in E1A that abolish its binding to pRB but leave the ability of E1A to transactivate viral genes intact. Due to the E1A mutation, the viruses are incapable of driving cells from G_0 and G_1 into S phase and replicate very poorly in quiescent or primary cells. They have been shown to replicate with great efficiency in tumour cells, however (Doronin *et al*., 2000). Other viruses bearing mutations that ablate the binding of E1A to pRB have also been reported. One such virus, designated *dl*922-947, was shown to possess superior cytopathic potency relative to ONYX-015 (Heise *et al*., 2000). Multiple intratumoural injections of another E1A mutant, termed Δ24, was

associated with an 83% reduction in tumour growth in a mouse model of glioma (Fueyo *et al.*, 2000).

Attempts to achieve tumour-selective expression of viral genes

An additional approach in an attempt to achieve tumour-specific virus-mediated cytolysis is to link virus replication to the transcription of cellular genes that are expressed in a tumour-specific manner. Rodriguez *et al.* (1997) attempted to create a prostate cancer-specific adenovirus by inserting sequences derived from the prostate-specific antigen (PSA) enhancer into the adenovirus genome so that they controlled expression of E1A. Replication of the CN706 virus was shown be much more efficient in cell lines expressing PSA than in those not expressing it. The same research group made a further modification to their original virus by inserting enhancer and promoter sequences derived from the prostate-specific kallikrein gene into the viral E1B gene (Yu *et al.*, 1999a). This virus, termed CV764, was shown to replicate very efficiently in PSA-expressing prostate cancer cell lines, whereas its replication in PSA-negative ovarian and breast cancer cells was very poor. Two further viruses were constructed by this group. In both of these viruses expression of the viral E1A and E1B genes was controlled by the prostate-specific rat probasin promoter and the prostate-specific PSA enhancer/promoters, respectively. The two viruses differed only in that the adenoviral E3 region was absent in one (CV739), whereas it was present in the other (CV787) (Yu *et al.*, 1999b). Studies showed that both viruses replicated in and lysed PSA-positive prostate cancer cells much more efficiently than PSA-negative cells and that the presence of the E3 region (which encodes the adenovirus 'death protein', as well as proteins important in evasion of the host immune response) significantly improved target cell killing and the efficacy in mouse xenograft models. These viruses are currently in clinical trials. Hallenbeck *et al.* (1999) reported constructing an adenovirus ('AvE1a04i') whose E1A is expressed from the tumour-specific α-fetoprotein (AFP) promoter. AFP is highly expressed in up to 80% of patients with hepatocellular carcinoma (HCC) but not in normal adults. The virus was demonstrated to replicate in AFP-expressing HCC cell lines, whereas little replication was observed in AFP-negative cell lines. More recently, Brunori *et al.* (2001) reported the construction of an adenovirus whose E1B and E2 genes are expressed from promoters controlled by the Tcf4 transcription factor. Tcf4 is constitutively active in virtually all colon tumours, whereas it is repressed in normal tissue. These researchers showed that viruses with Tcf4 regulation of E2 expression replicated normally in a colon cancer cell line but exhibited a 50- to 100-fold decrease in replication in lung cancer cells or normal fibroblasts, suggesting they may have potential in the treatment of colon carcinomas. Doronin *et al.* (2001) have engineered a significant degree of lung tissue-specificity into their already tumour-selective KD1 adenovirus (Doronin *et al.*, 2000) by replacing the viral E4 promoter with the promoter for the surfactant protein B gene, whose activity is restricted in adults to type II alveolar epithelial cells and brochial epithelial cells. In an attempt to achieve breast cancer-specific cytolyis, Kurihara *et al.* (2000) constructed two recombinant adenoviruses whose expression of E1A was controlled by the promoter of the *MUC1* gene. The *MUC1* gene is overexpressed in breast cancer cells and is transcriptionally regulated. Kurihara *et al.* (2000) demonstrated that the viruses selectively replicated in MUC1-positive breast cancer cells and that infection of human breast tumour xenografts in nude mice was associated with inhibition of tumour growth. One of the viruses (termed 'Ad.DF3-E1/CMV-TNF') was designed so as to express tumour necrosis factor (TNF) under the control of the human cytomegalovirus immediate early promoter. It was shown that infection with this virus was associated with selective replication and production of TNF in MUC1-expressing cells. Furthermore, treatment of MUC1-positive, but not MUC1-negative, breast cancer tumour xenografts with a single injection of Ad.DF3-E1/CMV-TNF was effective in inducing stable tumour regression in a mouse model. This showed not only that breast cancer selective replication could be achieved but that its anti-tumour activity is potentiated by an ability to express TNF. In the adult, albumin is expressed exclusively in the liver, so linking virus replication to albumin expression represents one strategy towards achieving liver-specific oncolysis. Miyatake *et al.* (1997) constructed a recombinant HSV (designated 'G92A') whose replication and cytotoxicity is limited to albumin-expressing cells by regulated expression of a viral immediate early protein. The genome of the ICP4-deleted HSV mutant d120 was modified by introducing an albumin enhancer/promoter–ICP4 transgene into the thymidine kinase gene. Studies showed that G92A replicated well in subcutaneous xenografts of human hepatoma cells but not in non-hepatoma tumours (Miyatake *et al.*, 1999). It should be noted that albumin is a tissue-specific molecule rather than a tumour-specific one and, as such, this approach has its limitations for treatment of primary liver cancer.

Translation of the non-capped RNA molecules that constitute the genomes of picornaviruses and flaviviruses is dependent upon the presence of an internal ribosomal entry site (IRES) element (reviewed by Martínez-Salas *et al.*, 2001). It has recently been shown that substitution of the IRES element in poliovirus by the corresponding element from another picornavirus, human rhinovirus type 2, yields a recombinant virus that is attenuated for neurovirulence in primates but replicates efficiently and lytically in cell lines derived from malignant gliomas (Gromeier *et al.*, 2000). Even though the apparent tumour specificity is likely to be due to differences in the efficiency of genome translation, this has yet to be confirmed.

Attempts to enhance the cytolytic effect of replication-competent viruses

(i) Expression of cytotoxic proteins

As stated earlier, adenoviruses express a nuclear membrane glycoprotein aptly termed the adenovirus death protein (ADP) very late in infection, which mediates efficient cell lysis and release of progeny virus from cells (Tollefson *et al*., 1996). The gene encoding this cytotoxic protein resides in the E3 region of the genome, which is deleted in the majority of adenovirus vectors constructed to date (Ring, 1996). Doronin *et al*. (2000) reintroduced the ADP gene back into the genome of an E3-deleted replication-competent adenovirus and demonstrated that ADP is expressed earlier in the replication cycle, and at higher levels than in natural infection. Furthermore, ADP-expressing viruses were shown to cause more rapid cell lysis than viruses from which ADP had been deleted. Thus, the removal of all the E3 region sequences, with the exception of the ADP gene, appears to result in an adenovirus that exhibits enhanced cytotoxicity.

It has recently been shown that expression of the membrane glycoproteins of measles virus and the gibbon ape leukaemia virus can kill cells by fusing them into large multi-nucleated syncytia (Bateman *et al*., 2000; Galanis *et al*., 2001). Measles virus has been shown to lyse human lymphoma cell lines *in vitro* and, indeed, to induce regression of human lymphoma xenografts in mice (Grote *et al*., 2001). It would be interesting to determine if the introduction of fusogenic membrane glycoprotein genes into the genomes of other replication-competent viruses would enhance the cytotoxicity of the virus. If so, such a modification could yield a virus with greater utility as an anti-cancer agent.

(ii) Expression of drug-sensitivity genes

One approach under development to increase the differential response of anti-cancer treatments between tumour and normal tissue (that is, the therapeutic index) is suicide gene therapy. This involves the transfer and expression of genes encoding enzymes that convert non-toxic pro-drugs into toxic antimetabolites (Springer & Niculescu-Duvaz, 2000). Three enzyme genes are currently being used in the context of replication-competent viruses. These are the genes encoding *Escherichia coli* cytosine deaminase, HSV-tk and the mammalian cytochrome P450 (CYP2B1), which confer sensitivity to 5-fluorocytosine, GCV and cyclophosphamide (CPA), respectively. The rationale behind the suicide gene therapy approach is that following transfer of these genes to the tumour site, only the tumour cells and neighbouring cells will be rendered sensitive to their cytotoxic action. Thus, with this approach, systemic toxicity commonly associated with, and a major limitation of, conventional chemotherapy, is minimized. A large number of studies have demonstrated the efficacy and safety of suicide gene therapy in animal models and it has been suggested that a combination of the cytolytic virus and suicide gene approaches may enhance the efficacy of each treatment. There appears to be some disagreement as to whether administration of GCV enhances or diminishes the cytolytic effects of HSV-tk-encoding viruses. For example, Aghi *et al*. (1999) and Freytag *et al*. (1998) reported that GCV treatment enhances the cytopathic effect of replication-competent virus, whereas Wildner & Morris (2000a, b) and Pawlik *et al*. (2000) reported that cytopathic effects are diminished. Treatment with GCV does, however, inhibit virus replication, thereby providing a means to control virus dissemination should viral treatment need to be terminated.

Chase *et al*. (1998) have constructed a recombinant HSV (designated 'rRp450') containing the CYP2B1 gene, which encodes the enzyme responsible for converting CPA to its anti-cancer metabolite phosphoramide mustard (Clarke & Waxman, 1989). The virus was deleted for the gene encoding ICP6, which is essential for virus replication and lysis of quiescent cells. The viral thymidine kinase gene remained intact, enabling replication of virus to be inhibited by GCV. Aghi *et al*. (1999) reported that human glioma cells infected with rRp450 exhibited a supra-additive sensitivity to both CPA and GCV. *In vivo*, regression of subcutaneous glioma xenografts in athymic mice was achieved by combined virus infection and CPA/GCV treatment. A therapeutic strategy based upon transfer of CYP2B1 by replication-defective virus vectors to endow tumour cells with oxazaphosphorine-susceptibility has also been shown to be successful (Wei *et al*., 1994; Chen & Waxman, 1995; Chen *et al*., 1997).

(iii) Expression of cytokine genes

The direct transfer of cytokine genes to tumour cells has emerged as a powerful immunotherapeutic tool in the new approaches to the management of cancer patients. In experiments with animal models, tumour cells transduced with cytokine and growth factor genes have demonstrated inhibition of tumour growth *in vivo* by stimulating inflammatory and immune responses (Oppenheim *et al*., 1997). In the light of this, it is likely that the anti-tumour effects of oncolytic virus would be enhanced by the inclusion of cytokine and growth factor genes in vector constructs. Zhang *et al*. (1996) constructed a replication-competent adenovirus with the consensus interferon gene inserted in the E3 region and showed that treatment of a human breast cancer xenograft with this virus was associated with rapid tumour regression. Moreover, a number of replication-competent recombinant HSVs that encode immunostimulatory molecules have been constructed in an attempt to enhance the destruction of tumours and induce anti-tumour immunity. Andreansky *et al*. (1998) described a virus ('R8306') in which both γ34.5 genes were replaced by a chimeric gene composed of the *Egr*-1 promoter and a murine interleukin (IL)-4 cDNA. They demonstrated that intracerebral inoculation of the IL-4-encoding virus into syngeneic murine glioma cells transplanted into the brains of immunocompetent

mice significantly prolonged survival of mice compared to control animals inoculated with saline. Furthermore, immunohistochemical analyses of mouse brains at 3 and 7 days after virus inoculation demonstrated marked accumulation of inflammatory cells composed of macrophages/microglia and $CD4^+$ and $CD8^+$ T cells.

Two groups have reported the construction of recombinant HSVs encoding murine IL-12 (mIL-12). Parker *et al*. (2000) used an intracranial syngeneic neuroblastoma mouse model and showed that median survival of mIL-12–virus-treated animals was significantly longer than the survival of animals treated with the parent virus lacking the mIL-12 gene. Furthermore, immunohistochemical studies demonstrated a profound influx of macrophages and $CD4^+$ and $CD8^+$ T cells into the tumours of mIL-12-treated mice compared to those treated with the control virus. Wong *et al*. (2001) described two replication-competent HSVs, one encoding murine granulocyte–macrophage colony-stimulating factor (GM-CSF; 'NV1034') and the other encoding mIL-12 ('NV1042'). In a squamous cell carcinoma VII (SCC VII) subcutaneous flank tumour model in immunocompetent mice, intratumoural injection of both viruses caused a significant reduction in tumour volume compared with saline injections; however, the NV1042-treated tumours showed a striking reduction in tumour volume compared to NV1034-treated tumours or tumours treated with the parent virus lacking cytokine genes ('NV1023'). Upon subsequent rechallenge, in the contralateral flank with SCC VII cells, only 43% of animals treated with the mIL-12 virus developed tumours compared to 86% of animals treated with the GM-CSF-encoding or cytokine-negative parent virus. This suggests not only that expression of IL-12 can potentiate oncolysis but that it may also induce a level of anti-tumour immunity.

Discussion

As many viruses lyse the cells in which they replicate, the suggestion that viruses might potentially be used to destroy specific cell populations is not altogether surprising. Since approximately 15% of the incidence of human cancer is attributable to virus infection (Weiss, 1998), it does, however, seem a little ironic that viruses might be used to combat cancer. As with any anti-cancer therapy, the cytotoxic effects of the treatment upon the normal tissue surrounding the tumour must be minimized. A number of viruses naturally replicate preferentially in transformed cells but it is unlikely that these viruses will demonstrate the degree of tumour selectivity required by the regulatory authorities for the routine treatment of cancer patients. Since naturally occurring viruses are unlikely to be deemed safe enough, the virus must be one that is amenable to genetic manipulation. The virus upon which the anti-cancer virus is based should ideally be one which is well characterized in terms of its replication in different cell types and its pathogenesis in the human population. The virus must be able to replicate efficiently in the context of a developing, or even a pre-existing antiviral immune response. This may require expression of viral proteins that are involved in suppression of the antiviral immune response. The virus must be able to disseminate throughout the tumour mass, destroying cells directly or sensitizing them to the action of other therapeutic agents, while sparing the surrounding normal tissue. Furthermore, infection with the virus should stimulate an effective anti-tumour immune response that would lead to the destruction of metastases. Pre-existing immunity could restrict the efficacy of any virus-mediated approach; however, it is noteworthy that the presence of antiviral antibodies did not diminished the anti-tumour effect of adenovirus ONYX-015 or measles virus when administered by the intratumoural route (Grote *et al*., 2001; Nemunaitis *et al*., 2000; Khuri *et al*., 2000). A number of articles report the potentiation of cytotoxic effects by viruses expressing drug-susceptibility genes while co-administrating the appropriate cytotoxic drug (Aghi *et al*., 1999; Chase *et al*., 1998; Freytag *et al*., 1998). Enhanced cytotoxic effects have also been achieved by the expression of IL-4 and IL-12. Moreover, anti-tumour immunity has been demonstrated following administration of an IL-12-expressing HSV (Wong *et al*., 2001). This is particularly noteworthy because stimulation of an anti-tumour immune response is likely to be critical to the long-term success of the treatment.

An 'ideal' anti-cancer virus would be based upon a highly lytic virus that has been modified so that it would only replicate in tumour cells (by manipulating the viral attachment proteins and/or the use of tumour-specific promoter/enhancer elements). Since different tumour types exhibit very different patterns of gene expression, it is likely that one will have to design a virus to each tumour type. Anti-cancer viruses are likely to be 'armed' with an number of genes that enhance their cytotoxicity. These would include genes encoding viral cytotoxic proteins, drug-susceptibility genes and cytokine genes. Viruses constructed to date exhibit one or more of these features; however, it is unknown whether all of these tumour-selectivity and cytotoxicity-enhancing features can be combined in one virus while maintaining replicative viability. Researchers should always be aware that manipulation of a virus, particularly its cellular tropism, could potentially lead to novel disease manifestations. Obviously all viruses will have to be thoroughly evaluated for safety, but the presence of a suicide gene such as HSV-tk in the virus adds confidence by allowing elimination of the virus should non-specific pathology arise. Even if replication-competent viruses are deemed sufficiently safe and efficacious in human clinical trials, it is unlikely that they will be used alone for the treatment of cancer. Indeed evidence from both pre-clinical and clinical studies suggests that combining replication-competent viruses with standard anti-cancer treatments such as chemotherapy and radiotherapy with result in greater therapeutic benefit (Freytag *et al*., 1998; Heise *et al*., 1997; Khuri *et al*., 2000; Rogulski *et al*., 2000).

Concluding remarks

The resistance of cancers to conventional treatments has stimulated the search for novel approaches. Replication-competent viruses offer great promise for cancer treatment because of their ability to amplify themselves and spread within the tumour mass. Furthermore, they are able to express foreign proteins that enhance their own inherent cytolytic potential. Significant progress has been made in targeting viruses to particular cell types, but a truly tumour-specific virus is yet to be constructed. It is likely that in the future a range of viruses that target different cells will become available for use in the fight against cancer.

Note added in proof. Bergmann *et al.* (2001) (*Cancer Research* **61**, 8188–8193, 2001) have recently demonstrated that an influenza A NS1 knockout virus replicates selectively in oncogenic *ras*-expressing cells and suggested that the virus represents an attractive candidate for the therapy of tumours exhibiting an activated *ras*-signalling pathway.

References

Aghi, M., Chou, T. C., Suling, K., Breakefield, X. O. & Chiocca, E. A. (1999). Multimodel cancer treatment mediated by a replicating oncolytic virus that delivers the oxazaphosphorine/rat cytochrome P450 2B1 and ganciclovir/herpes simplex virus thymidine kinase gene therapies. *Cancer Research* **59**, 3861–3865.

Ahlert, T. & Schirrmacher, V. (1990). Isolation of a human melanoma adapted Newcastle disease virus mutant with highly selective replication patterns. *Cancer Research* **50**, 5962–5968.

Akkina, R. K., Walton, R. M., Chen, M. L., Li, Q.-X., Planelles, V. & Chen, I. S. Y. (1996). High efficiency gene transfer into $CD34^+$ cells with a human immunodeficiency virus type 1-based retroviral vector pseudotyped with vesicular stomatitis virus envelope glycoprotein G. *Journal of Virology* **70**, 2581–2585.

Andreansky, S., He, B., van Cott, J., McGhee, J., Markert, J. M., Gillespie, G. Y., Roizman, B. & Whitely, R. J. (1998). Treatment of intracranial gliomas in immunocompetent mice using herpes simplex viruses that express murine interleukins. *Gene Therapy* **5**, 121–130.

Bateman, A., Bullough, F., Murphy, S., Emiliusen, L., Lavillette, D., Cosset, F.-L., Cattaneo, R., Russell, S. J. & Vile, R. G. (2000). Fusogenic membrane glycoproteins as a novel class of genes for the local and immune-mediated control of tumor growth. *Cancer Research* **60**, 1492–1497.

Bates, S. & Vousden, K. H. (1996). p53 in signaling checkpoint arrest or apoptosis. *Current Opinion in Genetics and Development* **6**, 12–18.

Bluming, A. Z. & Ziegler, J. L. (1971). Regression of Burkitt's lymphoma in association with measles infection. *Lancet* **ii, 105–106.**

Bolovan, C. A., Sawtell, N. M. & Thompson, R. L. (1994). ICP34.5 mutants of herpes simplex virus type I strains 17syn+ are attenuated for neurovirulence in mice and for replication in confluent primary mouse embryo cell cultures. *Journal of Virology* **68**, 48–55.

Boritz, E., Gerlach, J., Johnson, J. E. & Rose, J. K. (1999). Replication competent rhabdovirus with human immunodeficiency virus type I coats and green fluorescent protein: entry by a pH-independent pathway. *Journal of Virology* **73**, 6937–6945.

Boviatsis, E., Scharf, J., Chase, M., Harrington, K., Kowall, N. W., Breakfield, X. O. & Chiocca, E. A. (1994). Antitumor activity and reporter gene transfer into rat brain neoplasms inoculated with herpes simplex virus vectors defective in thymidine kinase or ribonucleotide reductase. *Gene Therapy* **1**, 323–331.

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

Brunori, M., Malerba, M., Kashiwazaki, H. & Iggo, R. (2001). Replicating adenoviruses that target tumors with constitutive activation of the wnt signaling pathway. *Journal of Virology* **75**, 2857–2865.

Burns, J. C., Friedmann, T., Driever, W., Burrascano, M. & Yee, J.-K. (1993). Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titre and efficient gene transfer into mammalian and nonmammalian cells. *Proceedings of the Society for Experimental Biology and Medicine* **90**, 8033–8037.

Cassel, W. A., Murray, D. R. & Phillips, H. S. (1983). A phase II study on the postsurgical management of stage II malignant melanoma with a Newcastle disease virus oncolysate. *Cancer* **52**, 856–860.

Chase, M., Chung, R. Y. & Chiocca, E. A. (1998). An oncolytic viral mutant that delivers the *CYP2B1* transgene and augments cyclophosphamide chemotherapy. *Nature Biotechnology* **16**, 444–448.

Chen, L. & Waxman, D. J. (1995). Intratumoural activation and enhanced chemotherapeutic effect of oxazaphosphorines following cytochrome P-450 gene transfer: development of a combined chemotherapy/cancer gene therapy strategy. *Cancer Research* **55**, 581–589.

Chen, L., Yu, L. J. & Waxman, D. J. (1997). Potentiation of cytochrome P450/cyclophosphamide-based cancer gene therapy by co-expression of the P450 reductase gene. *Cancer Research* **57**, 4830–4837.

Chou, J., Kern, E. R., Whitely, R. & Roizman, B. (1990). Mapping of herpes simplex virus neurovirulence to gamma 34.5, a gene nonessential for growth in culture. *Science* **250**, 1262–1266.

Clarke, L. & Waxman, D. J. (1989). Oxidative metabolism of cyclophosphamide: identification of the hepatic monooxygenase catalysts of drug activation. *Cancer Research* **49**, 2344–2350.

De Pace, N. (1912). Sulla scomparsa di un enorme cancro vegetante del collo dell'utero senza cura chirurgica. *Ginecologia* **9**, 82–89.

Dmitriev, I., Krasnykh, V., Miller, C. R., Wang, M., Kashentseva, E., Mikeeva, G., Belousova, N. & Curiel, D. T. (1998). An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. *Journal of Virology* **72**, 9706–9713.

Dorig, R. E., Marcil, A., Chopra, A. & Richardson, C. D. (1993). The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* **75**, 295–305.

Doronin, K., Toth, K., Kuppuswamy, M., Ward, P., Tollefson, A. E. & Wold, W. S. M. (2000). Tumor-specific, replication-competent adenovirus vectors overexpressing the adenovirus death protein. *Journal of Virology* **74**, 6147–6155.

Doronin, K., Kuppuswamy, M., Toth, K., Tollefson, A. E., Krajcsi, P., Krougliak, V. & Wold, W. S. M. (2001). Tissue-specific, tumor-selective, replication-competent adenovirus vector for cancer gene therapy. *Journal of Virology* **75**, 3314–3324.

Everett, H. & McFadden, G. (1999). Apoptosis: an innate immune response to virus infection. *Trends in Microbiology* **7**, 160–165.

Flanagan, A. D., Love, R. & Tesar, W. (1955). Propagation of Newcastle disease virus in Ehrlich ascites cells *in vitro* and *in vivo*. *Proceedings of the Society for Experimental Biology and Medicine* **90**, 82–86.

Flint, S. J., Enquist, L. W., Krug, R. M., Racaniello, V. R. & Skalka, A. M. (2000). *Principles of Virology: Molecular Biology, Pathogenesis and Control.* Washington, DC: American Society for Microbiology.

Freytag, S. O., Rogulski, K. R., Paielli, D. L., Gilbert, J. D. & Kim, J. H. (1998). A novel three pronged approach to kill cancer cells selectively: concomitant viral, double suicide gene, and radiotherapy. *Human Gene Therapy* **9**, 1323–1333.

Fueyo, J., Gomez-Manzano, C., Alemany, R., Lee, P. S. Y., McDonnell, T. J., Mitlianga, P., Shi, Y.-X., Levin, V. A., Yung, W. K. A. & Kyritsis, A. P. (2000). A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect *in vivo*. *Oncogene* **19**, 2–12.

Galanis, E., Bateman, A., Johnson, K., Diaz, R. M., James, C. D., Vile, R. & Russell, S. J. (2001). Use of viral fusogenic membrane glycoproteins as novel therapeutic transgenes in gliomas. *Human Gene Therapy* **12**, 811–821.

Ganly, I., Kirn, D. & Eckhardt, S. (2000). A phase I study of ONYX-015, an E1B attenuated adenovirus, administered intratumourally to patients with recurrent head and neck cancer. *Clinical Cancer Research* **6**, 798–806.

Goodrum, F. D. & Ornelles, D. A. (1998). P53 status does not determine outcome of E1b 55-kilodalton mutant adenovirus lytic infection. *Journal of Virology* **72**, 9479–9490.

Gromeier, M., Lachmann, S., Rosenfield, M. R., Gutin, P. H. & Wimmer, E. (2000). Intergenic poliovirus recombinants for the treatment of malignant glioma. *Proceedings of the National Academy of Sciences, USA* **97**, 6803–6808.

Grote, D., Russell, S. J., Corni, T. I., Cattaneo, R., Vile, R., Poland, G. A. & Fielding, A. K. (2001). Live attenuated measles virus induces regression of human lymphoma xenografts in immunodeficient mice. *Blood* **97**, 3746–3754.

Gu, D.-L., Gonzalez, A. M., Printz, M. A., Doukas, J., Ying, W., D'Andrea, M., Hoganson, D. K., Curiel, D. T., Douglas, J. T., Sosnowski, B. A., Baird, A., Aukerman, S. L. & Pierce, G. F. (1999). Fibroblast growth factor 2 retargeted adenovirus vector has redirected cellular tropism: evidence for reduced toxicity and enhanced antitumor activity in mice. *Cancer Research* **59**, 2608–2624.

Hallenbeck, P. L., Chang, Y.-N., Hay, C., Golightly, D., Stewart, D., Lin, J., Phipps, S. & Chiang, Y. L. (1999). A novel tumor-specific replication-restricted adenoviral vector for gene therapy of hepatocellular carcinoma. *Human Gene Therapy* **10**, 1721–1733.

Hammon, W. McD., Yohn, D. S., Casto, B. C. & Aitchison, R. W. (1963). Oncolytic potentials of nonhuman viruses for human cancer: effects of twenty-four viruses on human cancer cell lines. *Journal of the National Cancer Institute* **31**, 329–345.

Hammond, A. L., Plemper, R. K., Zhang, J., Schneider, U., Russell, S. J. & Cattaneo, R. (2001). Single-chain antibody displayed on a recombinant measles virus confers entry through the tumor-associated carcinoembryonic antigen. *Journal of Virology* **75**, 2087–2096.

Harada, J. N. & Berk, A. J. (1999). P53-independent and -dependent requirements for E1B-55K in adenovirus type 5 replication. *Journal of Virology* **73**, 5333–5344.

Hashiro, G., Loh, P. C. & Yau, J. T. (1977). The preferential cytotoxicity for reovirus for certain transformed cell lines. *Archives of Virology* **54**, 307–315.

Havenga, M. J., Lemckert, A. A., Grimbergen, J. M., Vogels, R., Huisman, L. G., Valerio, D., Bout, A. & Quax, P. H. (2001). Improved adenovirus vectors for infection of cardiovascular tissues. *Journal of Virology* **75**, 3335–3342.

Heise, C. & Kirn, D. H. (2000). Replication-selective adenoviruses as oncolytic agents. *Journal of Clinical Investigation* **105**, 847–851.

Heise, C., Sampson-Johannes, A., Williams, A., McCormick, F., Von Hoff, D. D. & Kirn, D. H. (1997). Onyx-015, an E1B-attenuated adenovirus causes tumor-specific lysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nature Medicine* **3**, 639–645.

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

Herold, B. C., Visalli, R. J., Sumarski, N., Brandt, C. R. & Spear, P. G. (1994). Glycoprotein C-independent binding of herpes simplex virus to cells requires cell surface heparan sulfate and glycoprotein B. *Journal of General Virology* **75**, 1211–1222.

Hutchin, M. E., Pickles, R. J. & Yarborough, W. G. (2000). Efficiency of adenovirus-mediated gene transfer to oropharyngeal epithelial cells correlates with cellular differentiation and human coxsackie and adenovirus receptor expression. *Human Gene Therapy* **11**, 2365–2375.

Jia, W. W., McDermott, M., Goldie, J., Cynader, M., Tan, J. & Tufaro, F. (1994). Selective destruction of gliomas in immunocompetent rats by thymidine kinase-defective herpes simplex virus type I. *Journal of the National Cancer Institute* **86**, 1209–1215.

Johnson, J. E., Schnell, M. J., Buonocore, L. & Rose, J. K. (1997). Specific targeting to CD4+ cells of recombinant vesicular stomatitis viruses encoding human immunodeficiency virus envelope proteins. *Journal of Virology* **71**, 5060–5068.

Kahn, J. S., Schnell, M. J., Buonocore, L. & Rose, J. K. (1999). Recombinant vesicular stomatitis virus expressing respiratory syncytial virus (RSV) glycoproteins: RSV fusion protein can mediate infection and fusion. *Virology* **254**, 81–91.

Kasono, K., Blackwell, J. L., Douglas, J. T., Dmitriev, I., Strong, T. V., Reynolds, P., Kropf, D. A., Carroll, W. R., Peters, G. E., Bucy, R. P., Curiel, D. T. & Krasnykh, V. (1999). Selective gene delivery to head and neck cancer cells via an integrin targeted adenoviral vector. *Clinical Cancer Research* **5**, 2571–2579.

Khuri, F. R., Nemunaitis, J., Ganly, I., Arseneau, J., Tannock, I. F., Romel, L., Gore, M., Ironside, J., MacDougall, R. H., Heise, C., Randlev, B., Gillenwater, A. M., Bruso, P., Kaye, S. B., Hong, W. K. & Kirn, D. H. (2000). A controlled trial of intratumoral ONYX-015, a selectively replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nature Medicine* **6**, 879–885.

Kirn, D., Nemunaitis, J., Ganly, I., Posner, M., Vokes, E., Kuhn, J., Heise, C., Maack, C. & Kaye, S. (1998). A phase II trial of intratumoural injection with an E1B-deleted adenovirus, ONYX-015, in patients with recurrent, refractory head and neck cancer. *Proceedings of the American Society of Clinical Oncology* **17**, 391.

Kirn, D., Martuza, R. L. & Zwiebel, J. (2001). Replication-selective virotherapy for cancer: biological principles, risk management and future directions. *Nature Medicine* **7**, 781–787.

Kramm, C. M., Chase, H., Herlinger, U., Jacobs, A., Pechan, P. A., Rainov, N. G., Sena-Esteves, M., Aghi, M., Barnett, F. H., Chiocca, E. A. & Breakefield, X. O. (1997). Therapeutic efficacy and safety of a second generation replication-conditional HSV-I vector for brain tumor gene therapy. *Human Gene Therapy* **8**, 2057–2068.

Kurihara, T., Brough, D. E., Kovesdi, I. & Kufe, D. W. (2000). Selectivity of a replication-competent adenovirus for human breast carcinoma cells expressing the MUC1 antigen. *Journal of Clinical Investigation* **106**, 763–771.

Laquerre, S., Anderson, D. B., Stoltz, D. B. & Glorioso, J. C. (1998). Recombinant herpes simplex virus type I engineered for targeted binding to erythropoietin receptor-bearing cells. *Journal of Virology* **72**, 9683–9697.

Levine, A. J. (1996). The origins of virology. In *Fields Virology*, pp. 1–14. Edited by B. N. Fields, D. M. Knipe & P. M. Howley. Philadelphia: Lippincott–Raven.

Lindblom, A. & Liljegren, A. (2000). Tumour markers in malignancies. *British Medical Journal* **320**, 424–427.

MacLean, A. R., Ul-Fareed, M., Robertson, L., Harland, J. & Brown, S. M. (1991). Herpes simplex virus type 1 deletion variants 1714 and 1716 pinpoint neurovirulence-related sequences in Glasgow strain 17^+ between immediate early gene 1 and the 'a' sequence. *Journal of General Virology* **72**, 631–639.

Marbert, J. M., Malick, A., Coen, D. M. & Martuza, R. L. (1993). Reduction and elimination of encephalitis in an experimental glioma therapy model with attenuated herpes simplex mutants that retain susceptibility to acyclovir. *Neurosurgery* **35**, 597–603.

Martínez-Salas, E., Ramos, R., Lafuente, E. & López de Quinto, S. (2001). Functional interactions in internal translation initiation directed by viral and cellular IRES elements. *Journal of General Virology* **82**, 973–984.

Martuza, R. L. (2000). Conditionally replicating herpes vectors for cancer therapy. *Journal of Clinical Investigation* **105**, 841–846.

Martuza, R., Malick, A. & Markert, J. M. (1991). Experimental therapy of human gliomas by means of a genetically-engineered virus mutant. *Science* **252**, 854–855.

Mineta, T., Rabkin, S. D. & Martuza, R. L. (1994). Treatment of malignant gliomas using ganciclovir hypersensitive ribonucleotide reductase deficient herpes simplex virus mutant. *Cancer Research* **54**, 3963–3966.

Mineta, T., Rabkin, S., Yazaki, T., Hunter, W. D. & Martuza, R. L. (1995). Attenuated multimutated herpes simplex virus type I for the treatment of malignant gliomas. *Nature Medicine* **1**, 938–944.

Miyatake, S.-I., Iyer, A., Martuza, R. L. & Rabkin, S. D. (1997). Transcriptional targeting of herpes simplex virus for cell-specific replication. *Journal of Virology* **71**, 5124–5132.

Miyatake, S.-I., Tani, S., Feigenbaum, F., Sundaresan, P., Toda, H., Narumi, O., Kikuchi, H., Hashimoto, N., Hangai, M., Martuza, R. L. & Rabkin, S. D. (1999). Hepatoma-specific anti-tumor activity of an albumin enhancer/promoter regulated herpes simplex virus in vivo. *Gene Therapy* **6**, 564–572.

Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, R. F., Rossi, B., Rabourdin-Combe, C. & Gerlier, D. (1993). Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *Journal of Virology* **67**, 6025–6032.

Nemerow, G. R. (2000). Cell receptors involved in adenovirus entry. *Virology* **274**, 1–4.

Nemunaitis, J., Ganly, I., Khuri, F., Arseneau, J., Kuhn, J., McCarty, T., Landers, S., Maples, P., Romels, L., Randlev, B., Reid, T., Kaye, S. & Kirn, D. (2000). Selective replication and oncolysis in p53 mutant tumors with Onyx-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. *Cancer Research* **60**, 6359–6366.

Nettelbeck, D. M., Jerome, V. & Muller, R. (2000). Gene therapy: designer promoters for tumour targeting. *Trends in Genetics* **16**, 174–181.

Nevins, J. R. (2001). The Rb/E2F pathway and cancer. *Human Molecular Genetics* **10**, 699–703.

Nevins, J. R. & Vogt, P. K. (1996). Cell transformation by viruses. In *Fields Virology*, pp. 301–343. Edited by B. N. Fields, D. M. Knipe & P. M. Howley. Philadelphia: Lippincott–Raven.

Oppenheim, J. J., Murphy, W. J., Chertox, O., Schirrmacher, V. & Wang, J. M. (1997). Prospects for cytokine and chemokine biotherapy. *Clinical Cancer Research* **3**, 2682–2686.

Parker, J. N., Gillespie, G. Y., Love, C. E., Randall, S., Whitely, R. J. & Markert, J. M. (2000). Engineered herpes simplex virus expressing IL-12 in the treatment of experimental murine brain tumors. *Proceedings of the National Academy of Sciences, USA* **97**, 2208–2213.

Pawlik, T. M., Nakamura, H., Yoon, S. S., Mullen, J. T., Chandrasekhar, S., Chiocca, E. A. & Tanabe, K. K. (2000). Oncolysis of diffuse hepatocellular carcinoma by intravascular administration of a replication-competent, genetically engineered herpesvirus. *Cancer Research* **60**, 2790–2795.

Phuangsab, A., Lorence, R. M., Reichard, K. W., Peeples, M. E. & Walter, R. J. (2001). Newcastle disease virus therapy of human tumor xenografts: antitumor effects of local or systemic administration. *Cancer Letters* **172**, 27–36.

Printz, M. A., Gonzalez, A. M., Cunningham, M., Gu, D.-L., Ong, M., Pierce, G. F. & Aukerman, S. L. (2000). Fibroblast growth factor 2-retargeted adenoviral vectors exhibit a modified biolocalization pattern and display reduced toxicity relative to native adenoviral vectors. *Human Gene Therapy* **11**, 191–204.

Pyles, R. B., Warnick, R. E., Chalk, C. L., Szanti, B. E. & Parysek, L. M. (1997). A novel multiply-mutated HSV-1 strain for the treatment of human brain tumors. *Human Gene Therapy* **8**, 533–544.

Ring, C. J. A. (1996). Adenovirus vectors. In *Gene Therapy*, pp. 61–76. Edited by N. R. Lemoine & D. N. Cooper. Oxford: Bios Scientific Publishers.

Ring, C. J. A. & Blair, E. D. (2000). Viruses as vehicles and expressors of genetic material. In *Genetically Engineered Viruses: Development and Applications*, pp. 1–4. Edited by C. J. A. Ring & E. D. Blair. Oxford: Bios Scientific Publishers.

Rodriguez, R., Schuur, E. R., Yeong Lim, H., Henderson, G. A., Simons, J. W. & Henderson, D. R. (1997). Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Research* **57**, 2559–2563.

Rogulski, K. R., Freytag, S. O., Zhang, K., Gilbert, J. D., Paielli, D. L., Kim, J. H., Heise, C. & Kirn, D. H. (2000). *In vivo* antitumor activity of ONYX-015 is influenced by *p*53 status and is augmented by radiotherapy. *Cancer Research* **60**, 1193–1196.

Rommelaere, J. & Tattershall, P. (1990). Oncosuppression by parvoviruses, pp. 41–57. In *Handbook of Parvoviruses*. Edited by P. Tijssen. Boca Raton, FL: CRC Press.

Rose, N. F., Roberts, A., Buonocore, L. & Rose, J. K. (2000). Glycoprotein exchange vectors based on vesicular stomatitis virus allow effective boosting and generation of neutralizing antibodies to a primary isolate of human immunodeficiency virus type 1. *Journal of Virology* **74**, 10903–10910.

Rothmann, T., Hengstermann, A., Whitaker, N. J., Scheffner, M. & Zur Hausen, H. (1998). Replication of ONYX-015, a potential anticancer adenovirus, is independent of p53 status in tumor cells. *Journal of Virology* **72**, 9470–9478.

Schneider, U., Bullough, F., Vongpunsawad, S., Russell, S. J. & Cattaneo, R. (2000). Recombinant measles viruses efficiently entering cells through targeted receptors. *Journal of Virology* **74**, 9928–9936.

Schnell, M. J., Buonocore, L., Kretzschmar, E., Johnson, E. & Rose, J. K. (1996). Foreign glycoproteins expressed from recombinant vesicular stomatitis viruses are incorporated efficiently into virus particles. *Proceedings of the National Academy of Sciences, USA* **93**, 11359–11365.

Schnell, M. J., Johnson, J. E., Buonocore, L. & Rose, J. K. (1997). Construction of a novel virus that targets HIV-1-infected cells and controls HIV-1 infection. *Cell* **90**, 849–857.

Shayakhmetov, D. M., Papayannopoulou, T., Stamatoyannopoulos, G. & Lieber, A. (2000). Efficient gene transfer into human CD34(+) cells by a retargeted adenovirus vector. *Journal of Virology* **74**, 2567–2583.

Shinoura, N., Yoshida, Y., Tsunoda, R., Ohashi, M., Zhang, W., Asai, A., Kirino, T. & Hamada, H. (1999). Highly attenuated cytopathic effect of a fiber-mutant E1B-defective adenovirus for gene therapy of gliomas. *Cancer Research* **59**, 3411–3416.

Sinkovics, J. & Horvath, J. (1993). New developments in the virus therapy of cancer: a historical review. *Intervirology* **36**, 193–214.

Sinkovics, J. & Horvath, J. (2000). Newcastle disease virus (NDV): a brief history of its oncolytic strains. *Journal of Clinical Virology* **16**, 1–15.

Spear, P. G. (1993). Entry of alphaherpesviruses into cells. *Seminars in Virology* **4**, 167–180.

Springer, C. J. & Niculescu-Duvaz, I. (2000). Pro-drug activating systems in suicide gene therapy. *Journal of Clinical Investigation* **105**, 1161–1167.

Steegenga, W. T., Riteco, N. & Bos, J. L. (1999). Infectivity and expression of the early adenovirus proteins are important regulators of wild-type and deltaE1B adenovirus replication in human cells. *Oncogene* **18**, 5032–5043.

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

Strong, J. E., Coffey, M. C., Tang, D., Sabinin, P. & Lee, P. W. K. (1998). The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus. *EMBO Journal* **12**, 3351–3362.

Suzuki, K., Fueyo, J., Krasnykh, V., Reynolds, P. N., Curiel, D. T. & Alemany, R. (2001). A conditionally-replicative adenovirus with enhanced infectivity shows improved oncolytic potency. *Clinical Cancer Research* **7**, 120–126.

Taqi, A. M., Abdurrahman, M. B., Yakubu, A. M. & Fleming, A. F. (1981). Regression of Hodgkin's disease after measles. *Lancet* **i, 1112.**

Tollefson, A., Scaria, A., Hermiston, T. W., Ryerse, J. S., Wold, L. J. & Wold, W. S. M. (1996). The adenovirus death protein (E3-11·6K) is required at late stages of infection for efficient lysis and release of adenovirus from infected cells. *Journal of Virology* **70**, 2296–2306.

Turnell, A. S., Grand, R. J. A. & Gallimore, P. H. (1999). The replicative capacities of large E1B-null group A and group C adenoviruses are independent of host cell p53 status. *Journal of Virology* **73**, 2074–2083.

Walther, W. & Stein, U. (2000). Viral vectors for gene transfer: a review of their use in the treatment of human diseases. *Drugs* **60**, 249–271.

Wei, M. X., Tamiya, T., Chase, M., Boviatsis, E. J., Chang, T. K. H., Hochberg, F. H., Waxman, D. J., Breakefield, X. O. & Chiocca, E. A. (1994). Experimental tumor therapy in mice with the cyclophosphamide-activating cytochrome P450 2B1. *Human Gene Therapy* **5**, 969–978.

Weiss, R. A. (1998). Introducing viruses and cancer. In *Viruses and Human Cancer*, pp. 1–15. Edited by J. R. Arrand & D. R. Harper. Oxford: Bios Scientific Publishers.

Wickham, T. J., Tzeng, E., Shears, L. L., II, Roelvink, P. W., Li, Y., Lee, G. M., Brough, D. E., Lizonova, A. & Kovesdi, I. (1997). Increased *in vitro* and *in vivo* gene transfer by adenovirus vectors containing chimeric fiber proteins. *Journal of Virology* **71**, 8221–8229.

Wilcox, M. E., Yang, W., Senger, D., Rewcastle, N. B., Morris, D. G., Brasher, P. M., Shi, Z. Q., Johnston, R. N., Nishikawa, S., Lee, P. W. & Forsyth, P. A. (2001). Reovirus as an oncolytic agent against experimental human malignant gliomas. *Journal of the National Cancer Institute* **93**, 903–912.

Wildner, O. & Morris, J. C. (2000a). The role of E1B 55 kDa gene product in oncolytic adenoviral vectors expressing herpes simplex virus tk: assessment of antitumor efficacy and toxicity. *Cancer Research* **60**, 4167–4174.

Wildner, O. & Morris, J. C. (2000b). Therapy of peritoneal carcinomatosis from colon cancer with oncolytic adenoviruses. *Journal of Gene Medicine* **2**, 353–360.

Wong, R. J., Patel, S. G., Kim, S.-H., DeMatteo, R. P., Malhotra, S., Bennett, J. J., St-Louis, M., Shah, J. P., Johnson, P. A. & Fong, Y. (2001). Cytokine gene transfer enhances herpes oncolytic therapy in murine squamous cell carcinoma. *Human Gene Therapy* **12**, 253–265.

Woods, D. B. & Vousden, K. H. (2001). Regulation of p53 function. *Experimental Cell Research* **264**, 56–66.

Yohn, D. S., Hammon, W. McD., Aitchison, R. W. & Casto, B. C. (1968). Oncolytic potentials of non-human viruses for human cancer: effects of five viruses on heterotransplantable human tumors. *Journal of the National Cancer Institute* **41**, 523–529.

Yu, D.-C., Sakamoto, G. T. & Henderson, D. R. (1999a). Identification of the transcriptional regulatory sequences of human kallikrein 2 and their use in the construction of calydon virus 764, an attenuated replication-competent adenovirus for prostate cancer therapy. *Cancer Research* **59**, 1498–1504.

Yu, D.-C., Chen, Y., Seng, M., Dilley, J. & Henderson, D. R. (1999b). The addition of adenovirus type 5 region E3 enables calydon virus 787 to eliminate distant prostate tumor xenografts. *Cancer Research* **59**, 4200–4203.

Zhang, J.-F., Hu, C., Geng, Y., Selm, J., Klein, S. B., Orazi, A. & Taylor, M. W. (1996). Treatment of a human breast cancer xenograft with an adenovirus vector containing an interferon gene results in rapid regression due to viral oncolysis and gene therapy. *Proceedings of the National Academy of Sciences, USA* **93**, 4513–4518.

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