Cytolytic viruses as potential anti-cancer agents

Christopher J. A. Ring

Gene Interference, GlaxoSmithKline Research & Development, Medicines Research Centre, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK

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

Author for correspondence: Christopher Ring.
Fax +44 1438 768097. e-mail cjr48991@gsk.com
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 rables 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; Boviatisis 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 αvβ3 and αvβ5. 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 than 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). Deficiency-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 un conjugated virus. More recently, Printz et al. (2000) have shown, using an intra-peritoneal 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 viruses. 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/IGFI 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 G0 and G1 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 G1/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 G1 arrest and apoptosis (Bates & Vousden, 1996). p53 is a transcription factor that mediates G1 arrest via induction of the p21WAF1/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 dl1520) 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 (Kirm et al., 1998; Ganly et al., 2000; Nemunaitis et al., 2000) and intra-tumoural 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 G0 and G1 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 dl922-947, was shown to possess superior cytopathic potency relative to ONYX-015 (Heise et al., 2000). Multiple intra-tumoural 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 to 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 bronchial epithelial cells. In an attempt to achieve breast cancer-specific cytolysis, 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 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 antitumour 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; Galinis 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 antimitabolites (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 diminish 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


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


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.


