Viral Therapy of Cancer

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Foreword

Cancer continues to represent a major global challenge despite advances made in the last 10 years that have seen improvements in survival rates for many of the common solid tumours. A number of cytotoxics, novel targeted agents, innovations in radiation oncology and new surgical techniques have been developed and all have played their part in the steady progress that has been made. However, some of the most important advances have come about due to better multidisciplinary working and successful multinational collaborations in clinical trials. Further work is required to optimize the standard anticancer modalities (surgery, radiotherapy, conventional chemotherapy and targeted agents) but even with the best efforts these are likely to yield little more than incremental gains in treatment outcomes.

The most significant change in oncology in the last 20 years has been our understanding of the molecular and genetic basis of cancer. In the early 1990s, this knowledge led to the development of an entirely new modality of treatment with a rationale based on fundamental molecular observations involving oncogenesis, immunology and intracellular signaling pathways. This new therapy was born out of the new biology, termed *gene therapy* and presented the biomedical community with the possibility of a quantum change in therapeutics. Suddenly there was the theoretical possibility of treating the root cause of a variety of diseases: not just cancer, but cardiovascular disorders, neurodegenerative conditions, inborn errors of metabolism and infectious diseases have all been the targets of this new therapeutic strategy.

Gene therapy represents the ultimate multidisciplinary activity. However, it should be regarded as a non-subject because it is more a series of scientific interdependencies coming together to achieve a particular therapeutic objective. *Viral Therapy of Cancer* illustrates this point very well with almost the entire gamut of bioscience and clinical expertise represented by the contributors. The book focuses on cancer and the use of viruses, both as vectors and as therapeutic agents, the latter strategy having grown out of the early days of gene therapy when viral vectors seemed to be the only possible way forward. The development of viral therapy demonstrates an important truth about gene therapy programmes: namely, that the field of gene therapy is not a strategy that should be judged simply by the triumphs or failures of clinical trials. It is a scientific activity of considerable consequence that spins out important scientific knowledge while at the same time making us question our current standard clinical trial methodologies which are not fit for all purposes, e.g. 'proof of principle' studies.

This book has been edited by three experts in the field of cancer gene therapy with experience of both laboratory and clinical research. The text bridges the gap between bench and bedside and will appeal to both basic scientists and clinicians with an interest in viral and gene therapy. The book is very comprehensive and deals with the biology, selectivity and clinical applications of the viruses that have been used as cancer therapeutics.

The multidisciplinary nature of gene therapy means that it is sometimes difficult for those involved; virologist, molecular biologist, clinician,

nurse, pharmacist, safety officer, to get accessible information about those areas of the activity in which that they are not expert. This book provides the reader with an excellent and comprehensive account of all aspects of the use of viruses as cancer therapy.

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Preface

Treatment modalities for cancer have expanded well beyond the traditional approaches of surgery, radiotherapy and chemotherapy. There has been an enormous surge of interest in the use of biological therapies, facilitated by a seismic shift in our understanding of the molecular basis of cancer. Although the first gene therapy trial using a retroviral vector was undertaken more than fifteen years ago, gene transfer therapy for cancer still awaits its first great breakthrough in terms of prolonging life. Having fairly recently confirmed the role of certain viruses in tumorigenesis, there appears to be a natural justice that we should now try and harness viruses for cancer therapy. Until recently, we would never have contemplated the use of replication-competent viruses for the treatment of cancer and, in fact, much of the early work in the field was deliberately restricted to the evolution of non-replicating viral vectors capable of efficient gene transfer. However, in 2008 the landscape has changed immeasurably and we are looking at the use of a wide range of replication-competent viruses as potential anti-cancer agents. These agents include those, that occur in nature and others that have been specifically engineered to have specific cytotoxicity against cancer cells, either as single agents or in combination with other anti-cancer modalities. The range of potential agents presents a variety of tropisms and individual strengths and weaknesses. Progress in this field has been astonishing in the last decade and as a result we felt that a comprehensive textbook coherently presenting the advances with the individual viruses was timely.

We have attempted to present a text which will appeal to the clinician, clinician-scientist and basic scientist as well as to allied health professionals. The chapters review the mechanistic and clinical background to a range of viral therapies and are designed to proceed from basic science at the bench to the patient's bedside to give an up-to-date and realistic evaluation of a therapy's potential utility for the cancer patient. We anticipate intense clinical activity in this arena in the next few years with a very real prospect that virotherapy may establish a role in the standard treatment of both common and rare cancers.

We thank Dr Kate Relph for her enormous contribution in the editing of this book.

This volume would not have been possible without the support of our families and, so, we wish to dedicate it: to Sindy, Simran and Savneet; to Memy, Oriana and Sebastian; to Katie and Lila Rose.

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1

Adenoviruses

Kate Relph, Kevin J. Harrington, Alan Melcher and **Hardev S. Pandha**

1.1 Introduction

Adenoviral vectors are the most popular vehicles for gene transfer currently being used in worldwide clinical trials for cancer. Over the past decade our knowledge of the adenoviral lifecycle together with the discovery of novel tumour antigens has permitted the targeting of adenoviral vectors to specific tumours. Targeting adenoviral vectors to tumours is crucial for their use in clinical applications in order to allow for systemic administration and the use of reduced vector doses. In addition, novel approaches to tumour killing have also been explored which will have greater potency and selectivity than currently available treatments such as chemotherapy or radiation. This chapter discusses the basic concepts behind the use of adenoviral vectors for cancer gene therapy, their potential for clinical application and where possible reviews ongoing and completed clinical trials.

1.2 Viral structure and life cycle

Adenoviruses are a frequent cause of upper respiratory tract infections and have also been associated with gastroenteritis and pneumonia in young children. They were first isolated in 1953 by scientists trying to establish cell lines from adenoidal tissue of children removed during tonsillectomy, and since then more than 50 different serotypes have been identified (Table 1.1) (Hilleman and Werner, 1954). The adenoviruses have been classified into six subgroups based on sequence homology and their ability to agglutinate red blood cells (Shenk, 1996). Most adenoviral vectors are derived from Ad2 or Ad5 which have been well studied and noted for their safety: over 50 per cent of the population show antibodies to adenovirus serotype 5 suggesting that it is particularly safe.

Adenovirus is a non-enveloped, icosahedral virus of about 60–90 nm in diameter with a linear double stranded genome of about 30–40 kb (Figure 1.1) (Stewart *et al*., 1993). The capsid consists of three major proteins, hexon (II), penton base (III), and a knobbed fibre (IV) along with a number of other minor proteins, VI, VII, IX, IIIa and IVa2. The virus genome has inverted terminal repeats (ITRs) and is associated with several proteins including a terminal protein (TP), which is attached to the 5′ end (Rekosh *et al*., 1977), a highly basic protein VII and a small peptide termed *mu* (Anderson *et al*., 1989). A further protein, V, links the DNA to the capsid via protein VI (Matthews and Russell, 1995).

The adenovirus life cycle essentially consists of the following steps. Virus entry into the cell is a two-stage process involving an initial interaction of the fibre protein with a range of cellular receptors, which include the major histocompatibility complex (MHC) class1 molecule and the coxsackie and adenovirus receptor CAR (Bergelson *et al*., 1997). The CAR is a plasma membrane protein of 46 kDa belonging to the immunoglobulin family

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Table 1.1 Adenoviral serotypes

Group	Serotypes
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35, 50
C	1, 2, 5, 6
D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51
E	4
F	40, 41

(Tomko *et al*., 1997). Some cell types, such as those of haematopoietic origin, do not express CAR on their cell surface and appear to be refractory to adenoviral infection (Mentel *et al*., 1997) suggesting that receptor recognition is one of the key factors in determining cell tropism. After initial interaction between the fibre knob and CAR the penton base protein then binds to the $\alpha_v\beta_3$ integrin family of cell surface heterodimers allowing internalization via receptor mediated endocytosis (Wickham *et al*., 1993). Penetration into the cell involves phagocytosis into phagocytic vesicles, after which the toxic activity of the pentons ruptures the phagocytic vacuoles and releases the vesicles into the cytoplasm. Release of the virus into the cytoplasm is accompanied by a stepwise dismantling of the capsid by proteolysis of protein VI (Greber *et al*., 1996). The partially dismantled viral particle is then delivered to the nucleus via microtubulin-assisted transport where the core-protein coated viral genome enters in through the nuclear pores.

Transcription of the adenoviral genome occurs in both early and late phases which occur before and after viral DNA replication respectively. A complex series of splicing events produces four early 'cassettes' of gene transcription termed E1, E2, E3 and E4 (Figure 1.2). The E1 proteins are divided into E1A and E1B. E1A is the first gene to

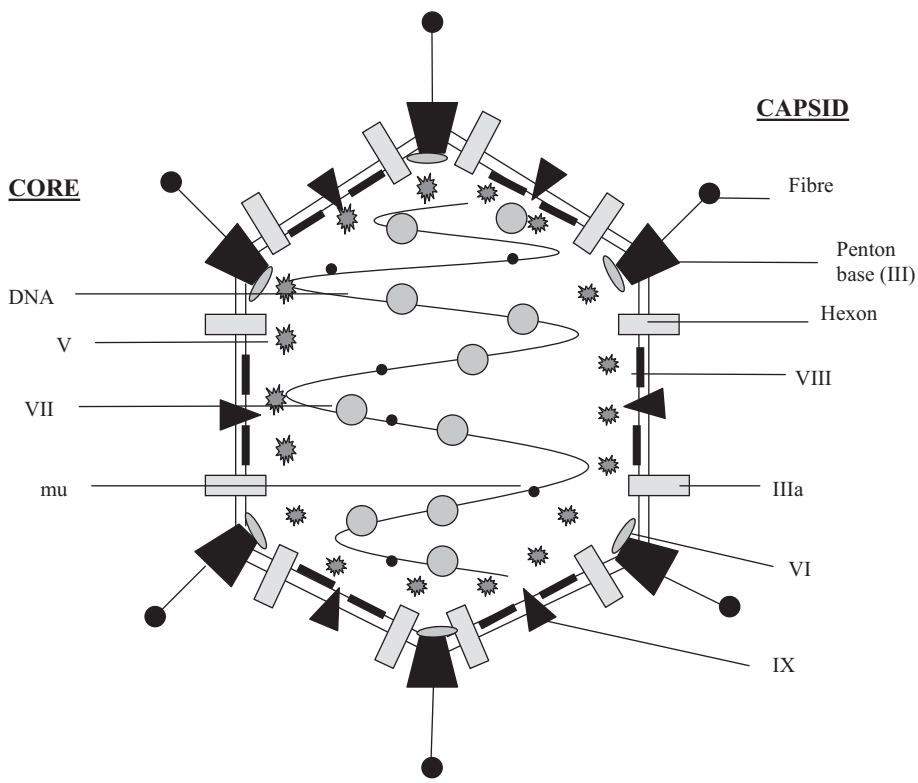

Figure 1.1 Structure of adenoviral capsid

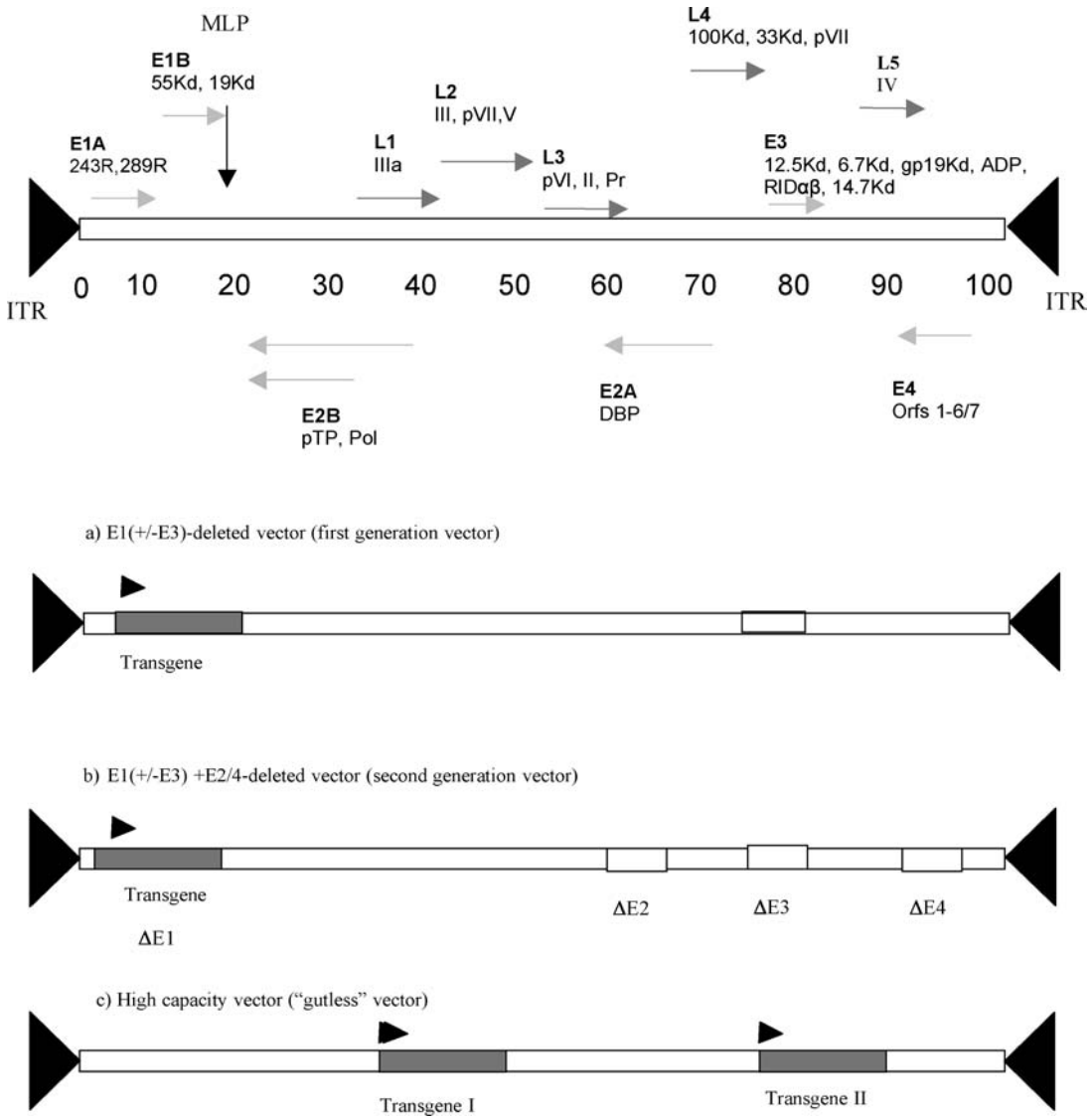

Figure 1.2 Schematic of adenoviral genome and adenoviral vectors. E1A must be removed to prevent recombinant virus from replicating. ITR, inverted terminal repeats

be expressed (Frisch and Mymryk, 2002) It encodes a transactivator for the transcription of the other early genes E1B, E2A, E2B, E3 and E4 but is primarily involved in many pathways to modulate cellular metabolism and make it more susceptible to viral replication (Table 1.2). E1A proteins interfere with cell division and regulation via direct and indirect action on a number of cellular proteins. For example E1A binds to the RB protein preventing it from binding to the transcription factor E2F. As a result E2F is transcriptionally active and can thus stimulate DNA synthesis. Also E1A maintains the stability of p53 via a variety of proteins and pathways including Mdm4, UBC9 and Sug1 (Table 1.2). E1A can directly bind and inhibit components involved in cell cycle control such as the cyclin dependent kinase inhibitor p21 (Chattopadhyay *et al.*, 2001).

Table 1.2 Some properties of E1A proteins

Property	Reference
Bind to p21 and related CDK inhibitors thereby stimulating cell division and growth	Chattopadhyay *et al.*, 2001
Bind to cyclins A and E-CDK complexes, which regulate passage to cell DNA synthesis	Faha *et al.*, 1993
Bind to the p300/CBP family of transactivators, which play a key role in regulating the transcription of many components of the cell cycle	Chakravati *et al.*, 1999
Binds to Rb and releases E2F- vital for synthesis of S-phase components as well as activation of E2 gene.	Brehm *et al.*, 1998
Interacts with multiprotein complex Sur-2, thereby stimulating the transcription of virus genes	Stevens *et al.*, 2002
Binds to the TATA-box binding protein to regulate transcription	Mazzarelli *et al.*, 1997
Induction of apoptosis via release of E2F which leads to increase in p53 and p19arf levels.	Hale and Braithwaite, 1999
Stabilises p53 via interaction with Sug1 a subunit of the proteasome complex that is required for proteolysis of p53	Grand *et al.*, 1999
Targets Mdm4 to stabilize tumour suppressor p53	Li *et al.*, 2004
Activates transcription of p73 and Noxa to induce apoptosis.	Flinterman *et al.*, 2005
Activates apoptosis by sensitizing cells to ionizing radiation, DNA damage, TNF and Fas ligand. Mediated by inhibiting the IκB kinases, which are critical for release of NFκB to nucleus and requires binding of E1A to P300/CBP	Shisler *et al.*, 1996
Binds to UBC9, a protein involved in the SUMO enzymatic pathway. Binding to E1A may interfere with SUMO modification of cellular proteins such as p53 and pRb	Desterro *et al.* 1999, Ledl *et al.* 2005

NFκB, nuclear factor κB.

It can also interact with a number of host factors involved in mediating chromatin structure including p400 (Fuchs *et al.*, 2001) and the histone acetyl transferases p300, pCAF and TRRAP/GCN5 (Lang and Hearing, 2003). Other early gene products are also involved in making the cell more refractory to viral replication. The E1B 19K protein is analogous to the Bcl-2 gene product and is concerned with increasing cell survival and ablating members of the Bax family which induce apoptosis (Han *et al.*, 1996). A second 55 kDa protein product of the E1B gene has been shown to interact with p53 reducing its transcription. The E1b protein has also been shown to block host mRNA transport to the cytoplasm (Pilder *et al.*, 1986). The E2 gene encodes proteins required for viral DNA replication, i.e. DNA polymerase, DNA-binding protein and the precursor of the terminal protein (de Jong *et al.*, 2003). Despite replicating in the nucleus the adenovirus need its own enzymatic machinery because of its complex chromosomal structure. The genome lacks telomeres and so the integrity of the ends of the DNA is maintained by a viral preterminal protein which is covalently linked to the 5′ end and acts as a primer for the viral DNA polymerase. The E3 genes encode a variety of transcripts involved in subverting the host defence mechanism (Wold and Chinnadurai, 2000). The E3-gp19K protein acts to prevent presentation of viral antigens by MHC class I pathway and therefore blocks cell lysis by cytotoxic T cells (Bennett *et al.*, 1999). One E3 protein is termed the adenovirus death protein (ADP) as it facilitates late cytolysis of the infected cell and thereby releases progeny virus more efficiently (Tollefson *et al.*, 1996a). The E4 proteins

mainly facilitate virus mRNA metabolism and promote virus DNA replication and shut off of host protein synthesis (Halbert *et al*., 1985).

Replication of the viral genome starts about 5–6 h after infection and is dependant on the inverted terminal repeats (ITRs) which act as the origins of replication. Adenovirus DNA replication has been studied extensively both *in vivo* (t.s. mutants in infected cells) and *in vitro* (nuclear extracts). At least three virus-encoded proteins are known to be involved in DNA replication: TP acts as a primer for initiation of synthesis. Ad DBP – a DNA-binding protein and Ad DNA Pol – 140 kDa DNA-dependent polymerase. The onset of DNA replication signals the pattern of transcription changes from early to late genes and only newly replicated DNA is used for late gene transcription. Late phase transcription is driven primarily through the major late promoter with five transcripts resulting from a complex series of splicing events. These transcripts are mainly used for the production of viral structural proteins. Encapsidation of the virus depends on the presence of a packaging signal near the 5′ end of the genome consisting of an AT-rich sequence. Intranuclear virion assembly starts about 8 h after infection and leads to the production of 10^4 to 10^5 progeny particles per cell, which can be released after final proteolytic maturation by cell lysis 30–40 h post-infection, completing the viral life cycle (Shenk, 1996).

1.3 Adenoviral vectors

Adenoviral vectors are attractive reagents for gene therapy because of their ability to transduce genes into a broad range of cells, and to infect both dividing and non-dividing cells (McConnell and Imperiale, 2004). Adenoviral vectors can accommodate large segments of DNA (up to 7.5 kb) and the viral genome rarely undergoes rearrangement meaning that inserted genes are maintained without change during virus replication. In addition, adenoviruses replicate episomally and do not insert their genome into that of the host cell ensuring less disruption of vital cellular genes and processes and reduced risk of insertional mutagenesis. This can, however, be a limitation in that transient expression of the therapeutic gene may be inadequate to treat chronic conditions such as cystic fibrosis. However, for situations in which short-term activity of the gene is needed, such as expression of suicide genes selectively in tumour cells, these viruses are suitable vectors. The adenoviral genes can be separated into two groups; the *cis*-genes, such as those responsible for the packaging signal, which must be carried by the virus itself, and the *trans*-genes which can generally be complemented and therefore replaced with ‘foreign’ DNA. The first generation of adenoviral vectors were used for the delivery of genes in monogenic disorders (Figure 1.2a). In these vectors the E1 region was removed to inhibit viral replication and make way for the therapeutic gene. Many of the first generation vectors also contained a deletion in the E3 region in order to allow for even greater transgenes to be incorporated. The E3 genes are dispensable for virus growth *in vitro* but some data suggests that E3 genes in vectors may be beneficial *in vivo* due to their ability to dampen the immune response (Bruder *et al*., 1997). However, despite the removal of these regions of the viral genome there was still low-level transcription of viral genes, which led to a host cellular immune response and a reduction in the period of gene expression due to cell-mediated destruction of the transduced cells (Kay *et al*., 1995; Yang *et al*., 1995). In addition these types of vectors allowed the generation of E1 containing replication competent adenovirus (RCA) due to homologous recombination in 293 cells which further enhanced the adverse effects (Lochmuller *et al*., 1994). In order to address these problems homologies between the vectors and the complementing cell lines have been reduced. Second generation adenoviral vectors have further deletions in E2a, E2b or E4 and have reduced immunogenicity and RCA generation (Figure 1.2b). Despite these improvements the complementing cell lines are difficult to engineer, can be difficult to grow and can lead to poor viral titers (Lusky *et al*., 1998). As a result a third generation of adenoviral or gutless vectors have been created (Parks *et al*., 1996) (Figure 1.2c). These have all of the viral genes deleted (except for the packaging signal) and replaced with the therapeutic gene of interest. They are therefore free from problems associated with immunogenicity

and demonstrate long-term transgene expression. They are generated with a helper virus, which contains all of the genes necessary for viral replication but which contains a deletion in the packaging signal to ensure that it is not incorporated into the final vector. These vectors are still undergoing development in order to improve their purity and large-scale manufacture (Wu and Attai, 2000).

1.4 Targeting adenoviral vectors

Despite the fact that adenoviral vectors have many advantages over other gene transfer vehicles there are some problems associated with their use. The broad tropism of adenoviral vectors as well as being an advantage also represents an important limitation for their use in therapeutic applications. Animal studies have shown that adenoviral vectors do not remain confined to one compartment and are able to disseminate to distal sites with toxic effects that are most notable in the liver (Wang *et al*., 2003; Yee *et al*., 1996). This also restricts the systemic administration of the vectors due to the potential for toxicity in normal tissues (Brand *et al*., 1997). In addition, important target tissues are often refractory to adenoviral infection leading to administration of increased doses of vector in an attempt to improve gene transfer. This in turn often leads to increased toxicity and enhanced humoral and cellular immune responses. Clearly there is a requirement for targeted adenoviral vectors in clinical applications in order to allow for systemic administration and the use of reduced vector doses, which will in turn reduce inflammatory, and immune responses (Mizuguchi and Hayakawa, 2004). Two main approaches have been taken in order to target expression of the therapeutic gene to the required tissue/tumour: (1) transductional targeting and (2) transcriptional targeting.

1.4.1 Transductional targeting of adenoviral vectors

The identification of the route by which human cells uptake adenovirus was an important step towards retargeting adenoviral vectors to different cell types, also known as transductional targeting. The adenovirus fibre knob anchors onto the surface of the target cell by means of the CAR and interaction of the capsid penton protein with integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ on the surface of target cells allows internalization (Bergelson *et al*., 1997; Wickham *et al*., 1993). Most immortalized tumour cell lines express CAR and are therefore easily transduced by adenoviral vectors. However, certain studies have demonstrated that 50 per cent of primary epithelial cancers do not express CAR (Kasono *et al*., 1999; Vanderkwaak *et al*., 1999). This may account for some of the limited success with past clinical trials using adenoviral vectors. Transductional targeting may improve transfer of genes to particular cancer types, such as glioma, and in addition retargeting adenoviral vectors will permit the treatment of haematological malignancies because haematopoietic stem cells are known to lack CAR (Huang *et al*., 1996).

There are many reports of retargeting of adenoviral vectors to tumour cells via the use of antibodies directed towards specific antigens on the surface of a particular tumour type (Barnett *et al*., 2002). One group used a neutralizing anti-fibre antibody conjugated to an antibody directed against the epithelial cell adhesion molecule (EGP-2), which is highly expressed on the surface of a range of adenocarcinomas from the stomach, oesophagus, breast, ovary, colon and lung and its expression is limited in normal tissue. In this study the adenovirus specifically infected cancer cell lines expressing EGP-2 whilst gene transfer was dramatically reduced in EGP-2-negative cell lines. A recent study combines genetic ablation of native adenoviral tropism with redirection of viral binding to melanoma cells via a bispecific adaptor molecule (Nettelbeck *et al*., 2004). This molecule consists of a bacterially expressed single chain diabody, scDb MelAd that binds to both the adenoviral fibre knob and to the high molecular weight melanoma associated antigen (HMWMAA), which is widely expressed on the surface of melanoma cells. This retargeting strategy mediated up to a 54-fold increase in adenoviral gene transfer to CAR-negative melanoma cells compared to a vector with native tropism.

Further targeting has been achieved by altering the structure of the fibre knob itself by inserting an arginine-glycine-aspartate (RGD) tripepetide

(Buskens *et al*., 2003). Four oesophageal carcinoma cell lines and ten fresh surgical resection specimens were cultured and infected with either native adenovirus or retargeted adenovirus expressing the luciferase gene or green fluorescent protein to analyse gene transfer efficiencies. In both the cell lines and the primary cells more efficient gene transfer was seen with the retargeted virus. This phenomenon was less pronounced in normal cells.

1.4.2 Transcriptional targeting of adenoviral vectors

The targeting of gene expression to specific cell types/tissues can be achieved through the use of tumour or tissue specific promoters. This approach has been adopted in a range of studies targeting gene expression to tumours (Rots *et al*., 2003; Haviv and Curiel, 2001). A recent study identified the cyclooxygenase-2 (cox-2) gene as a potential new target for melanoma gene therapy (Nettelbeck *et al*., 2003). An adenoviral vector was constructed in which the cox-2 promoter drove the expression of a luciferase reporter gene. Melanoma cell lines, primary melanoma cells and normal melanocytes were infected with this novel vector. The results demonstrated activity of the cox-2 promoter in the melanoma cell lines and primary melanoma cells but not in non-malignant primary epidermal melanocytes. Several approaches have also considered the use of two different tumour specific promoters within the same vector in order to achieve a further degree of specificity. The second promoter is normally one that is a more general promoter which shows activity in a broad range of tumours such as the telomerase reverse transcriptase promoter.

In suicide gene therapy for cancer (discussed later) targeting is paramount to prevent unwanted toxicity. For example, the product of the thymidine kinase gene itself, without addition of the prodrug ganciclovir, has been shown to cause liver toxicity when under the control of the cytomegalovirus promoter (Yamamoto *et al*., 2001). Several groups have therefore engineered adenoviral vectors to contain tissue/tumour specific regulatory elements in order to avoid these problems and target toxicity specifically to the transduced cells. One study used the prostate specific antigen promoter to target expression of HSV-TK to benign prostatic hyperplasia (Park *et al*., 2003). This approach induced highly selective and definite ablation of epithelial cells in benign canine prostate.

Both transcriptional and transductional targeting have improved the efficacy of adenoviral vectors significantly. Some groups are now investigating the possibilities of combining these two approaches to further improve the specificity of adenoviral vectors. For example a combination of the tissue-specific SLP1 promoter and the ovarian cancer associated targeting adaptor protein, sCARfC6.5, which contains the CAR ectodomain and a single-chain antibody specific for c-erbB-2, increased the efficacy and specificity of adenoviral gene therapy for ovarian carcinoma (Barker *et al*., 2003.

1.5 Clinical applications of adenoviral gene therapy

Advances in adenoviral vector technology have meant that there are now 140 clinical trials worldwide currently being conducted on various cancers using adenoviral vectors (*Journal of Gene Medicine* www.wiley.co.uk/wileychi/genemed). Table 1.3 gives details of seventeen completed gene therapy trials for cancer using adenoviral vectors. All of theses were phase I studies to test toxicity. Table 1.4 indicates some of the ongoing clinical phase II trials. Several approaches have been used to destroy the target tumour cells:

1.6 Adenoviral vectors for immunotherapy

T lymphocytes play a crucial role in the host's immune response to cancer. Although there is ample evidence for the presence of tumour-associated antigens on a variety of tumours, they are often unable to elicit an adequate antitumour response. Our increasing knowledge of the cellular interactions required to induce a specific antitumour response has led to the development of cancer vaccines which prime the host response and induce or enhance T-cell reactivity against tumour antigens. Gene-based strategies for

Table 1.3 A selection of completed phase I clinical trials using adenoviral vectors for the treatment of cancer

Investigator	Country	Cancer	Gene	No. of patients	Reference
Stewart	Canada	Breast, melanoma	IL-2	23	Stewart *et al*., 1999
Tursz	France	Non-small cell lung carcinoma	Il-2	21	Griscelli *et al*., 2003
Tursz	France	Non small cell lung carcinoma	Beta-gal	21	Griscelli *et al*., 2003
Eck	USA	CNS	HSV-TK	N/C	N/C
Reid	USA	Anaplastic thyroid cancer	p53	N/C	N/C
Roth	USA	Non-small cell lung carcinoma	p53	N/C	N/C
Belani	USA	Hepatocellular carcinoma	p53	N/C	N/C
Belldegrun	USA	Prostate	p53	N/C	N/C
Hasenburg	Germany	Ovarian	HSV-tk	10	Hasenburg *et al*., 2002
Kauczor	Germany	Non-small cell lung carcinoma	p53	6	Kauczor *et al*., 1999
Fujiwara	Japan	Non-small cell lung carcinoma	p53		Fujiwara *et al*., 1999
Boulay	Switzerland	Non small cell lung carcinoma	p53	N/C	N/C
–	Switzerland	Metastases from solid tumours	IFNγ	N/C	N/C
Albertini	UK	Melanoma	IFNγ	N/C	N/C
Lafollette	UK	Head and neck carcinoma	E1b del.	N/C	N/C
Lafollette	UK	Ovarian	E1b del.	16	Vasey *et al*., 2002
Stewart	UK	Gastrointestinal cancer	p53	N/C	N/C

N/C = not stated.
Source: *Journal of Gene Medicine* website (http:www.wiley.co.uk/wileychi/genmed)