REVIEW

Gene therapy strategies for the treatment of pituitary tumours

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INTRODUCTION

Gene therapy can be defined as the introduction of therapeutic nucleic acids into cells with the aim of ameliorating or curing a disease. Initially, gene therapy paradigms focused on single-gene disorders. Recently, it has become apparent that potential therapeutic nucleic acids can be considered as drugs and as such could also be used for treating a variety of diseases (Verma & Somia 1997, Lowenstein 1997). With recent advances in gene transfer technology, gene therapy has become a reality, with more than 2100 patients currently undergoing clinical gene therapy trials (Marcel & Grausz 1997). Given the high expectations from this new therapeutic modality and in spite of the lack of conclusive evidence for its efficacy from the initial trials, very valuable information has been gained from the preclinical and clinical trials.

There are two main methods for transferring therapeutic transgenes into affected tissues: transplantation of autologous cells genetically modified *ex vivo* and direct delivery of the therapeutic transgenes into the target tissue. As far as gene therapy methods are concerned, *ex vivo* methods are losing popularity in relation to *in vivo* approaches (59% of patients by December 1994, and 39% of patients by December 1995) (Marcel & Grausz 1997).

Currently, there are two major approaches for transferring therapeutic genes into diseased cells: using viral and non-viral vectors. Viral vectors used for clinical gene therapy include retroviruses, herpes simplex type 1 viruses (HSV1), adeno-associated viruses (AAVs), lentiviruses and adenoviruses (Kozarsky & Wilson 1993, Verma & Somia 1997). Non-viral vectors include naked DNA, liposomes and DNA-protein complexes (Kay *et al.* 1997) (Table 1). The therapeutic potential of each approach needs to be evaluated individually, taking

into account various factors, e.g. nature of the disease to be treated, desired duration of treatment, number and type of cells to be transduced, level of transgene expression required.

RETROVIRAL VECTORS

Retroviruses are a group of RNA viruses in which the genome is reverse-transcribed to double-stranded DNA in the infected cell. The genome comprises three genes named *gag*, *pol* and *env*, which encode viral structural proteins, reverse transcriptase and integrase, and the virion envelope protein respectively. The viral genome is flanked by long terminal repeats (LTRs) which mediate integration into the host genome and control expression of the viral genes, i.e. they act as promoter-enhancer sequences. The packaging sequence (Ψ) mediates the packaging of the genomic-length RNA into virions and distinguishes viral RNA from cellular RNAs (Varmus *et al.* 1979, Bender *et al.* 1987, Brown *et al.* 1989).

Retroviral-based vectors currently used for gene delivery are replication-defective because of the deletion of the *gag*, *pol* and *env* genes. These functions are provided in trans using specially designed packaging cell lines, so that all that is needed to generate infectious virions are the LTRs and the packaging sequence (Ψ) (Miller 1992). Transcription of the gene of interest can be driven by the viral LTRs; alternatively, other promoter/enhancer elements can be introduced together with the transgene. The recombinant retroviral vector is introduced into the appropriate packaging cell line, which produces all necessary viral proteins in trans, and therefore the culture medium contains the retroviral vectors. One of the main limitations of retroviral vectors is that they cannot infect non-dividing cells (Miller *et al.* 1990). They are

TABLE 1. Characteristics of gene transfer vectors

Property	Retrovirus	Lentivirus	HSV1	Adenovirus	AAV	Liposomes/: naked DNA
Maximum insert size (kb)	7-7.5	7-7.5	~35	~30	3.5-4.0	No limitation
Concentration (viral particles per ml)	~10 ⁸	~10 ⁸	~10 ⁸	~10 ¹²	~10 ¹²	No limitation
Integration	Yes	Yes	No	No	Yes/No	Poor
Stability	Good	Good	Good	Good	Good	Very good
Immune response	Low	Low	Medium	Extensive	Not known	None
Pre-existing immune-response	Unlikely	Unlikely	Yes	Yes	Yes	No
Safety	Insertional mutagenesis	Insertional mutagenesis	Inflammation, toxicity	Inflammation, toxicity	Inflammation, toxicity	None

efficient at transducing cells in culture, but, taking into account that *in vivo* most cells are quiescent at any one time, this hampers the use of retrovirus vectors for *in vivo* gene transfer/therapy applications. Another disadvantage of retroviruses is that the virus titres that can be achieved are relatively low (10⁷-10⁸ plaque-forming units/ml). Also, the possibility of random integration of vector DNA into the host genome could lead to activation of oncogenes or disruption of tumour suppressor genes, although theoretically this possibility is almost negligible. To date, retroviruses based on the mouse Moloney leukaemia virus have been used most frequently in clinical trials (Marcel & Grausz 1997).

More recently, a chimaeric Moloney-human lentiviral (human immunodeficiency virus (HIV)) vector has been generated which can transduce non-dividing cells such as neurons in the central nervous system of rodents (Naldini *et al.* 1996a). Like other retroviruses, HIV has the three *gag*, *pol* and *env* genes, but it also carries genes that encode six accessory proteins, i.e. *tat*, *rev*, *vpr*, *vpu*, *nef* and *vif*.

Lentivirus vectors have been constructed with the transgene of interest inserted between the LTRs and packaging sequence (Naldini *et al.* 1996b). Some of the accessory genes can be excised without affecting vector production or infection efficiency (Naldini *et al.* 1996b). Infection *in vivo* in a variety of tissues like the brain, liver, muscle or pancreas has shown up to 6 months sustained expression (Blomet *et al.* 1997, Miyoshi *et al.* 1997). Unlike with classical retroviral vectors, 'shut off' of transgene expression has not been observed. There is little information available on possible immune reactions to lentiviral vectors, but no cellular immune response has been detected at the site of infection in the brain (Naldini *et al.* 1996b). These vectors can be produced at titres of 10⁹ virus particles/ml. A challenge with these vectors is to guarantee their safety for gene therapy applications in humans.

ADENOVIRAL (AD) VECTORS

Human adenoviruses cause mild upper respiratory tract infections, and the two most common serotypes (Ad2 and Ad5) are currently being developed as vectors for gene transfer and gene therapy. These adenoviruses are not oncogenic in humans, and live adenovirus vaccines have been effectively used in humans. Compared with other gene transfer systems available, adenovirus-based systems achieve the highest levels of gene expression per unit DNA. Furthermore, they are the only

gene transfer system that can survive bloodstream delivery.

The basic characteristics of this virus have been extensively reviewed (Shenk 1996). The adenovirus genome is composed of linear double-stranded DNA approximately 36 kbp long, divided into 100 map units (mu) of 360 bp each. The viral genome contains short inverted terminal repeats (ITRs) at the 5' and 3' ends which are needed for viral DNA replication. Gene expression is divided into early (E1 through E4) and late (L1 through L5) regions, according to when expression takes place, i.e. before or after viral DNA replication. The adenoviral genome does not usually integrate into the host DNA; they are replicated as episomal elements within the nucleus of the target cell.

The replication-deficient recombinant adenovirus (RAV) vectors developed for gene transfer/gene therapy applications carry deletions of the entire E1a and part of the E1b regions. In this way, space is made to insert therapeutic expression cassettes, and also, in the absence of the E1a gene product, the virus cannot replicate. Therefore no viral spread occurs after infection of target cells unless wild-type virus is present to complement the vectors.

Replication-defective adenoviruses are grown in cells that express the products of the E1 gene, i.e. stably transfected 293 cells, where very high viral titres can be obtained ($\sim 10^{11}$ - 10^{12} infectious adenovirus particles per ml) (Lowenstein *et al.* 1996). Variations on the procedure for generating RAVs have been described including additional deletions in the E3 and E4 regions and, more recently, deletions of all viral genes, 'gut-less' vectors, which contain only the elements that define the start and finish of the viral genome, and the viral packaging signal (Parks *et al.* 1996).

Several features make replication-deficient adenoviruses very attractive vectors for gene therapy applications, i.e. they can be easily generated and concentrated to high titres ($\sim 10^{12}$ plaque-forming units/ml), they can transduce both dividing and non-dividing cells, they do not integrate into the host chromosomal DNA, and they are safe. These vectors have been used to infect a wide range of cells and tissues both *in vitro* and *in vivo*, eliciting high levels of transgene expression (Bajocchi *et al.* 1993, Freese *et al.* 1996, Geddes *et al.* 1996, 1997, Brown *et al.* 1997, Castro *et al.* 1997, Shering *et al.* 1997).

One of the main drawbacks of RAVs for gene therapy applications is the limited duration of transgene expression after *in vivo* delivery. Transgene expression is extended in nude rats and mice or if the RAV is delivered together with immunosuppressants (Zsengeller *et al.* 1995, Geddes *et al.* 1996, Jooss *et al.* 1996, Hermens & Verhaagen 1997,

Ilan *et al.* 1997). The immune reaction elicited by RAVs can be evidenced by an early inflammatory response, which eliminates most of the initial viral load, and also a later cellular and humoral immune response (Wood *et al.* 1996). Therefore virally infected cells are eventually killed by cytotoxic lymphocytes, and the humoral immune response will generate specific antibodies to adenoviral proteins, preventing any subsequent readministration of the virus. In spite of these problems, adenoviruses are extremely useful vectors, if expression of therapeutic transgenes is needed for limited periods of time, i.e. for cancer gene therapy applications in which the aim is to kill the tumour cells in the shortest possible time, and inflammatory responses can be beneficial. One widely used approach is to deliver genes that can cause tumour cell killing or immune stimulating genes that will activate the immune response of the host specifically towards tumour cells. In these cases, the cellular immune response to RAV proteins should result in increased tumour cell death (Smith *et al.* 1997).

AAV VECTORS

AAVs are non-pathogenic single-stranded DNA viruses. The two genes, i.e. *cap* (capsid) and *rep* (replication), are flanked by ITRs which are located at the extreme ends of the AAV genome and contain the packaging sequence. The *cap* gene encodes the viral capsid proteins and the *rep* gene product is involved in viral replication and integration. AAVs need further genes to replicate which are provided by a helper virus such as adenovirus or HSV.

AAVs have recently been shown to transduce brain (McCown *et al.* 1996), liver (Snyder *et al.* 1997) and skeletal muscle (Xiao *et al.* 1996, Fisher *et al.* 1997). In the presence of the *rep* gene product, the viral DNA can integrate preferentially into human chromosome 19. The *rep* and *cap* genes are replaced with a transgene of interest to produce an AAV vector which normally has a cloning capacity of 3.5-4.0 kb of foreign DNA. Thus larger cDNAs or complex regulatory elements cannot be expressed using this vector. Preparation of AAV vectors is laborious, and up to 10^{11} - 10^{12} viral particles/ml can be obtained, but only one in 100 to 1000 particles will be infectious. To date there are no packaging cell lines available and therefore vector preparations have to be carefully purified of contaminating adenoviruses used as helpers. Also, the immunogenicity of AAVs needs to be investigated further, especially in view of the fact that 80% of the adult population has circulating antibodies to AAVs.

OTHER VIRAL VECTORS

A number of other viral vectors are currently being developed. HSV1 can infect and establish long-term latent infections in neuronal cells. Therefore it is an attractive vector for performing gene therapy for neurological disorders. The HSV1 genome is large (150 kb encoding ~80 genes) and can be difficult to manipulate, but a rich source of mutants and genetic information is available. Around 10^8 – 10^9 viral particles/ml can be produced, but residual proteins are toxic to target cells (Lowenstein *et al.* 1994). Several genes can be deleted, allowing a larger cloning capacity. One of the main limitations of this vector system is the toxicity of recombinant HSV1 vectors for long-term gene expression to prolong neuronal cell survival in diseases such as Parkinson's; problems to be solved are how to avoid inducing an inflammatory response and how to deliver the virus to specific brain regions.

Vaccinia-virus-derived vectors are currently being used, mainly for generating vaccines (Moss 1996), i.e. for tumour treatment. The Sindbis and Semliki Forest virus are being developed as cytoplasmic vectors which are not translocated into the nucleus (Berglund *et al.* 1993). A number of additional viral vectors based on Epstein–Barr virus, Simian virus 40, papilloma virus, non-human lentiviruses and hepatitis viruses are currently being developed by several groups. It is likely that chimaeric viruses will have the most desired properties for clinical gene therapy applications.

NON-VIRAL DELIVERY SYSTEMS: LIPOSOMES AND NAKED DNA

Naked plasmid DNA has been used as a gene delivery system both *in vitro* and *in vivo*. Direct infection of naked plasmid DNA into muscle has resulted in transfection levels similar to those obtained *in vitro* for fibroblasts (Wolff *et al.* 1990). Although plasmid DNA does not integrate into the host genome, expression of the transgene is long lived (Jiao *et al.* 1992, Davis *et al.* 1993). It has been hypothesised that uptake of DNA and long-term transgene expression within skeletal and cardiac muscle is due to the fact that the transverse tubules of the myofibres are in direct contact with the extracellular space and this makes DNA uptake very efficient (Acsadi *et al.* 1991). However, use of naked plasmid DNA for the treatment of genetic diseases of skeletal muscle would require efficient transfection and adequate levels of transgene expression within a large proportion of muscle tissue. Another possible application is the expression of transgenes

in muscle for the development of vaccines (Fuller & Haynes 1994, Lowrie *et al.* 1994, Webster *et al.* 1994).

Another approach to the delivery of genetic material within target cells is to encapsulate the plasmid DNA within a liposomal carrier. Two types of cationic lipids have been developed; the first has two alkyl chains in each cationic lipid molecule and the second uses cholesterol as the backbone. They both contain one or several amino groups which form complexes with the plasmid DNA through electrostatic interactions. In general, the transfection efficiency of these cationic lipids is improved when another lipid, e.g. dioleoylphosphatidylethanolamine, is incorporated (Felgner *et al.* 1994). In principle, cationic-lipid-based DNA delivery systems offer several advantages, i.e. they are less immunogenic, the DNA–lipid complexes are easy to prepare, there is no limit to the size of the transgenes that can be delivered, and they have less risk of generating tumorigenic mutations since plasmid DNA has very low integration frequency and cannot replicate. One disadvantage of this system is that it has much lower transgene expression potency and transduction efficiency when compared with viral delivery systems. This implies that the toxicity associated with cationic-lipid-based delivery systems becomes an important issue to be considered. As with viral delivery systems, future developments should be aimed to achieve cell-type-specific transgene expression and delivery, tightly regulated levels of transgene expression and increased length of transgene expression.

GENE THERAPY STRATEGIES FOR THE TREATMENT OF PITUITARY TUMOURS

Pituitary tumours account for approximately 10–15% of all cranial tumours (Thapar *et al.* 1997). Although they are often considered as 'benign' adenomas, they produce distressing endocrine abnormalities, they can often exhibit suprasellar extensions into the cranium, and, depending on the size of the tumour, ophthalmoplegia and other neurological symptoms may be present. Owing to recent progress in endocrine assays, imaging techniques, transsphenoidal microsurgery, receptor-mediated pharmacology and radiotherapy, most pituitary tumours have become manageable disorders, with long-term survival or cure of the affected individuals. Nevertheless, for some of the most difficult tumours, such as certain large and locally invasive endocrine-active tumours, only partial success has been possible, and, in such cases,

cure might prove impossible to achieve. The pathogenesis and molecular biology of pituitary adenomas, as well as the effectiveness of the currently used treatment strategies, have been extensively reviewed elsewhere (Molitch 1989, Wilson 1990, Bevan *et al.* 1992, Adams & Burke 1993, Braba *et al.* 1993, Levy & Lightman 1993, 1994, Shimon & Melmed 1997). Therefore I will only outline possible gene therapy strategies that could be applicable for the treatment of pituitary tumours that prove resistant to conventional treatment strategies. Some of these strategies have been or are currently being developed for the treatment of other cancers, e.g. brain tumours, colon cancer, liver metastasis, breast cancer, both in animal models and in human clinical trials (Marcel & Gausz 1997).

Use of prodrug-activating genes

The most commonly used prodrug-activating enzyme gene is the thymidine kinase (*tk*) gene from HSV1 (Moolten 1986). HSV1-*tk* converts nucleoside analogues, e.g. acyclovir and ganciclovir, into their phosphorylated metabolites which then act as competitive inhibitors of endogenous nucleotides for incorporation into replicating DNA chains of proliferating cells, causing cell death. HSV1-*tk* can mediate ganciclovir tumour cell killing both *in vitro* and *in vivo* (Moolten 1994). The effectiveness of this approach can be enhanced by the 'bystander' effect in which non-transduced cells can be killed by the transfer of phosphorylated ganciclovir metabolites through gap junctions (Freeman *et al.* 1993, Vile & Hart 1993). This strategy is currently being used for many gene therapy trials for brain tumours and also other peripheral cancers. Other commonly used genes that activate prodrugs include: the *Escherichia coli* cytosine deaminase (*cd*) gene which activates 5-fluorocytosine to the cytotoxic agent 5-fluorouracil, the *E. coli* guanine phosphoribosyl-transferase (*gpt*) gene which activates 6-thioxanthine and 6-thioguanine, the *E. coli* nitroreductase gene, the mammalian deoxycytidine kinase gene which activates cytosine arabinoside, the rat cytochrome P450 2B1 gene which activates cyclophosphamide/ ifosphamide, and the bacterial carboxypeptidase G2 gene which activates 4-(N,N-bis(2-iodoethyl) amino) phenoxycarbonyl L-glutamic acid (CMDA) (reviewed by Moolten 1994, Chiocca 1998).

A recombinant adenovirus vector expressing HSV1-*tk* under the control of a truncated major immediate early human cytomegalovirus promoter (MIEhCMV) has been recently generated and used to induce *in vitro* cell killing of anterior pituitary corticotrophic and prolactotrophic tumour cells, i.e. AtT20 and GH3 respectively (Dewey *et al.*

1997, Windeatt *et al.* 1998). These results suggest that these RADs could be used effectively to achieve gene therapy for pituitary tumours. Moreover, the RAD/*tk* was found to be non-cytotoxic to the surrounding normal pituitary tissue, suggesting that this treatment would not affect the functioning of the normal anterior pituitary gland.

Use of tumour suppressor genes and genes that regulate the cell cycle

Tumour suppressor genes normally suppress cell proliferation. Both copies of the genes need to be mutated in order to initiate oncogenic transformation. The mutated or deleted p53 suppressor gene is associated with 50% of human cancers. However, to date, p53 mutations have not been detected in either secreting or non-secreting pituitary adenomas, carcinomas or their metastases (Levy *et al.* 1994, Shimon & Melmed 1997). Therefore p53 probably plays no major role in pituitary tumorigenesis. A recent study demonstrated that deletions affecting chromosomes 11q13, 13q12-14 and 10q26 occur to a significantly greater extent in invasive than non-invasive pituitary tumours (Bates *et al.* 1997). 11q13 deletions have been reported in up to 30% of sporadic pituitary tumours (Bates *et al.* 1997). These observations suggest that tumour suppressor gene interaction is involved in tumour progression and, once identified, they could become possible targets for gene therapy strategies. Pituitary adenomas can also develop as part of multiple endocrine neoplasia type 1 (MEN1). The gene responsible for MEN1 has recently been cloned (Chandrasekharappa *et al.* 1997). Mutations and allelic deletions at the precise location of the MEN1 genes were investigated in sporadic pituitary adenomas; the results did not show any mutations or deletions of the MEN1 gene in the majority of these tumours (Zhuang *et al.* 1997). Thus this tumour suppressor gene is probably not a possible target for gene therapy in non-MEN1 tumours (i.e. the vast majority).

The retinoblastoma (Rb) gene product regulates the cell cycle and plays an important role in regulating cell differentiation. It has recently been demonstrated that heterologous disruption of the Rb gene in mice leads to 100% incidence of pro-opiomelanocortin-expressing pituitary tumours which originate from the intermediate lobe of the pituitary gland (Hu *et al.* 1994). Similarly, mice with mutations in the p27^{kip1} gene, often develop pituitary tumours (Nakayama *et al.* 1996). In humans, it has been suggested that another tumour suppressor gene located in chromosome 13q adjacent to the Rb locus, and not the Rb gene itself,

may play a role in the development of invasive pituitary adenomas and carcinomas (Shimon & Melmed 1997). Recently a gene therapy strategy has been successfully used *in vivo* for the treatment of intermediate lobe pituitary tumours (Riley *et al.* 1996). Adenoviruses encoding the Rb cDNA have been used to treat spontaneous pituitary melanotroph tumours arising in immunocompetent Rb+/- mice. Intratumour delivery of RAd/Rb decreased tumour cell proliferation, re-established innervation by dopaminergic neurons which regulate cell growth, inhibited tumour growth, and prolonged the life spans of treated animals. This study therefore provides good proof of the amenability of this gene therapy strategy for the treatment of pituitary tumours, although it would need to be adapted by using other tumour suppressor genes useful for human therapeutic applications.

Growth factors and their receptors involved in modulating angiogenesis

The process of angiogenesis occurs in many physiological conditions, but it also plays a major role in the sustained growth of solid tumours. It has also been shown that angiogenesis is essential for the growth of oestrogen-induced prolactin-secreting anterior pituitary tumours (Elias & Weiner 1984). It was recently demonstrated that fumagillin and its analogue TNP-470, which are known to selectively inhibit endothelial cell proliferation, inhibited prolactin production and prolactotrophic cell density and proliferation and suppressed neovascularisation in Fischer 374 rats bearing prolactinomas induced by oestrogen treatment (Takachi *et al.* 1994, Stepien *et al.* 1996). These results provide strong evidence for the anti-prolactinoma effects of inhibitors of angiogenesis in this pituitary prolactinoma model. Therefore the process of neovascularisation of expanding pituitary adenomas offers a target for experimental gene therapy approaches based on inhibiting angiogenesis. Several growth factors and their respective receptors are known to be involved in the process of angiogenesis and have been shown to be necessary for tumour growth. These include vascular endothelial growth factor (VEGF) (Samoto *et al.* 1995) and its receptor (VEGFR-1 or flt-1 and VEGFR-2 or flk-1) (Milauer *et al.* 1994), transforming growth factor α (TGF α) and its receptor, epidermal growth factor (EGF) and its receptor, TGF β , human platelet factor 4 (PF4) and its receptor (Maione *et al.* 1991) and basic fibroblast growth factor (bFGF) and its receptor (Nabel *et al.* 1993).

Concerning pituitary tumorigenesis, it has been shown that EGF and its receptor (EGFR) are

present in human pituitary adenomas, and the levels of EGFR correlate with tumour aggressiveness (LeRiche *et al.* 1996). High molecular mass forms of bFGF have also been identified and characterised in human pituitary adenomas (Li *et al.* 1992). Normal human pituitary tissue and secreting and non-secreting adenomas can also express TGF α (Driman *et al.* 1992, Ezzat *et al.* 1995). Moreover, a lactotroph-targeted TGF α -overexpressing transgenic mouse exhibited hyperplasia of prolactotrophic cells and developed prolactinomas (McAndrew *et al.* 1995). Gene therapy strategies involving anti-angiogenic genes may provide an effective *in vivo* therapy, i.e. expression of antisense cDNAs for bFGF, EGF or VEGF, or the expression of dominant negative mutants of their respective receptors. These strategies have been successfully used *in vivo* for treatment of brain tumours in animal models (Milauer *et al.* 1994, Redekop & Naus 1995, Saleh *et al.* 1996).

OTHER GROWTH FACTORS

Nerve growth factor (NGF) is a neurotrophic protein that promotes the growth, differentiation and survival of neurons of the peripheral sympathetic nervous system, the ascending cholinergic neurons of the basal forebrain and sensory nerve cells derived from the neural crest (Levi-Montalcini 1987, Higgins *et al.* 1989). It has also been reported that endocrine cells are responsive to NGF (Missale *et al.* 1991). In a recent study, the same group reported that human prolactinomas that were totally resistant to pharmacological therapy because they lacked D-2 receptors for dopamine expressed NGF receptors (Missale *et al.* 1993). They also demonstrated that, after exposure to NGF, tumour cells decreased their proliferation rate, lost their capacity to form colonies in soft agar, lost their tumorigenic capacity in nude mice and re-expressed the D-2 receptor. These results indicate that short-term treatment with NGF may induce the reversion of human prolactinomas to a more differentiated less malignant dopamine-sensitive prolactotroph-like state (Missale *et al.* 1993). This study raises the possibility that a short-term gene therapy strategy, based on the delivery of NGF to the tumour *in situ*, may restore the susceptibility of refractory tumours to conventional treatment with D-2 agonists.

CONCLUSION

The events that mediate viral entry into cells and the expression of transgenes encoded within the

viral genome have been the subject of intensive research during the last decade. This, coupled with the interest in developing techniques that allow gene transfer and expression within mammalian cells and tissues, has led to the development and use of modified viruses as vectors. Efficient transgene expression within all cell types of the anterior pituitary gland with no apparent cytotoxicity has recently been achieved using RAd vectors (Castro *et al.* 1997). These RAds have also been successfully used for gene transfer into mouse (Riley *et al.* 1996), rat (Castro *et al.* 1997) and human pituitary tumour cells (Freese *et al.* 1996) both *in vitro* and *in vivo*. It is also possible to engineer cell-type-specific and regulatable expression cassettes within recombinant adenovirus vectors for the expression of therapeutic genes within a specific cell population of the anterior pituitary gland which can also be turned 'on' and 'off' as required using the tetracycline-inducible system (Gossen *et al.* 1994, Massie *et al.* 1998). Furthermore, it is now feasible to engineer adenoviral vectors with a determined tropism and to produce them at high titres and purity. This, in combination with carefully designed and controlled clinical trials, should enable the assessment of whether virus-mediated gene therapy could become a useful strategy for the treatment of currently difficult-to-treat pituitary tumours.

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