Gene therapy for pituitary tumours

J R E Davis¹, P R Lowenstein², A S McNeilly³ and M G Castro²

¹Endocrine Sciences Group, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

²Molecular Medicine Unit, Clinical Research Division II, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

³MRC Reproductive Biology Unit, University of Edinburgh, Edinburgh, UK

(Requests for offprints should be addressed to J R E Davis; Email: julian.davis@man.ac.uk)

Introduction

Pituitary tumours in man are an unusual type of neoplasm. Although small pituitary tumours are common, affecting as many as 20% of the population, clinical problems are relatively rare and the tumours are generally indolent, with slow growth over many years. Nonetheless, they may cause major clinical problems from mass effects (causing headache and visual failure from optic chiasm compression) and hormone hypersecretion from functioning tumours, most commonly prolactin excess from prolactinomas. Major advances have been made in the therapy of pituitary tumours over the past 20 to 30 years, but despite this their treatment often remains unsatisfactory in practice. There is, therefore, a place for improvements in therapy and in this review we will outline the potential development and application of adenoviral gene therapy for treatment of human pituitary tumours.

Clinical background

Pituitary tumours that cause clinical problems can be classified according to their patterns of hormone production and their clinical presentation. Thus, tumours can be described as 'functioning', in which overproduction of prolactin, growth hormone, adrenocorticotrophin (ACTH), gonadotrophins or thyrotrophin may cause a clinical syndrome, or 'non-functioning', in which no clinical syndrome is apparent, although some of these tumours produce gonadotrophin subunits. Pituitary tumours are unusual in that they are normally highly differentiated and slow growing. Their differentiated nature means that they often retain many of the characteristics of the normal cell type, including the regulatory mechanisms controlling hormone production.

Lactotroph tumours are the commonest type of functioning pituitary tumour, and the prevalence of hyperprolactinaemia in the population may be as high as 0.5% (Miyai *et al.* 1986). Growth hormone (GH)-secreting somatotroph tumours are much rarer, and the acromegaly that they cause affects about forty people/million in Western Europe. Cushing's disease, caused by

corticotroph tumours secreting ACTH and other pro-opiomelanocortin precursor peptides, is also rare. Non-functioning tumours comprise 40% of all pituitary tumours, and perhaps partly because they produce no clinical syndrome apart from eventual hypopituitarism, they tend to be large by the time of presentation.

All these types of tumours may present as microadenomas, defined as tumours less than 1 cm in diameter and contained within the pituitary fossa, or as macroadenomas that have extended outside the fossa to invade adjacent tissues (from 1 cm to over 10 cm in diameter). Tumour extension outwith the pituitary fossa may involve the cavernous sinus laterally (parasellar extension), the optic nerve superiorly (suprasellar extension), or the sphenoid sinus inferiorly. Rarely, aggressive tumours may extend along the skull base, even sometimes causing multiple cranial nerve palsies (e.g. Davis *et al.* 1990), but only exceptionally are they truly malignant with distant metastasis. Macroadenomas commonly come to clinical attention through pressure effects such as headache or progressive visual failure, whereas microadenomas are usually identified through investigation of a clinical endocrine syndrome.

Current therapy for pituitary tumours - limitations

The aims of any therapy for pituitary tumours depend on the clinical presentation, but in general the aim of treatment will be to reduce tumour mass and to reduce hormone excess. Debulking of significant tumour mass is important to reduce symptoms of headache from compression of surrounding structures, and particularly to relieve pressure on the optic nerve from suprasellar extension. However, normalisation of the endocrine abnormality is coming to be seen as equally important for long-term health and even long-term mortality, thus an ideal therapy should also reduce hormone hypersecretion to normal or 'safe' levels (see, for discussion relating to acromegaly, Bates *et al.* 1993, Sheppard 1994, Orme *et al.* 1998).

Surgery offers the potential for long-term cure by total excision of a pituitary adenoma, leaving intact the remaining normal pituitary gland. In the treatment of microadenomas, trans-sphenoidal pituitary microadenomectomy is safe and carries low risks of damage to the normal pituitary. Nonetheless, in a recent series of patients with GH-secreting tumours operated on by a specialist surgeon, 14% of patients developed new pituitary dysfunction as a result of the surgery, and 7% were left with permanent diabetes insipidus (Ahmed *et al.* 1999). Fistula formation with cerebral spinal fluid leakage (2% rate in this series) may require further surgery in some cases; meningitis can occur but is now rare. Surgery for macroadenomas is often essential for debulking and relief of pressure symptoms, but as the tumours are larger, there is often a greater risk of damage to related structures. Many tumours can be treated by the trans-sphenoidal route, but some require transfrontal craniotomy, which carries greater operative risks.

Despite a generally satisfactory safety record, however, surgery carries surprisingly low cure rates for the endocrine abnormality, and the results are poorer the larger the tumour. Published surgical series generally reflect the results of highly specialized centres, but even so, the cure rates are disappointingly low. For microprolactinoma, for example, different surgical series report long-term rates of overall endocrine 'cure' varying between 45% (Soule *et al.* 1996) and 73% (Thomson *et al.* 1994), and a meta-analysis of surgical series in the USA reported overall cure rates of only 53% (Molitch *et al.* 1997). For prolactin-secreting macroadenomas, the rates of endocrine cure are much worse, at best only 13-17% (Bevan *et al.* 1992). Similar results have been reported for treatment of acromegaly: the overall endocrine success rate in a recent series was 67%, but this depended greatly on the size of the tumour and also improved with increasing experience of the surgeon (Ahmed *et al.* 1999). Results from non-specialist pituitary surgeons are likely to be poorer than those reported in the literature, as suggested by a recent audit of results for acromegaly (Lissett *et al.* 1998). In summary, therefore, surgery has potential for cure and therefore remains a first choice of therapy in many circumstances, but the actual outcomes are often disappointing, even in specialists' series.

Medical therapy has brought about a revolution in our expectations of treatment for pituitary disease. In many cases, drug treatment alone can prove adequate without recourse to any other therapy. Key medical therapies include dopamine agonists for hyperprolactinaemia, and somatostatin analogues and growth hormone antagonists for acromegaly, and their current status will be briefly considered.

The astonishing success of dopamine agonists in both reducing serum prolactin levels and causing shrinkage of

prolactinomas has allowed drugs such as bromocriptine, cabergoline and quinagolide to be used as sole therapy for many patients with hyperprolactinaemia (Bevan *et al.* 1992). Despite their success in 85-90% of patients, however, they have a high rate of side effects, notably nausea and vomiting, postural hypotension and dizziness, headache and constipation. Depressive reactions may also be seen in some patients. At least 20% of patients experience significant nausea while taking bromocriptine, and even the less nauseating drug, cabergoline, had to be stopped by 3% of patients in a double-blind trial (Webster *et al.* 1994). Thus, these drugs, although remarkably effective, are often disliked by patients and sometimes cannot be used.

Somatostatin analogues do not cause clinically useful shrinkage of somatotroph tumours, but they frequently reduce GH levels, if not to normal, at least to levels that appear to be 'safe' in terms of normalising long-term mortality (Sheppard 1994). Their clinical use has been much eased by the introduction of depot preparations of octreotide and lanreotide that can be given at 2- to 4-week intervals, but there is still a requirement for uncomfortable repeated injections over long periods of time, and treatment can involve great expense over many years. Growth hormone antagonists are under clinical trial at present, and may be an important advance, although they are unlikely to affect the size or growth of the underlying pituitary tumour. So far they appear to be well tolerated, although information on side-effects is likely to emerge from current trials. For small tumours, both somatostatin analogues and GH antagonists may find a place as sole therapy for acromegaly, although frequently the size of the pituitary tumour also necessitates surgical treatment.

Pituitary irradiation is effective in reducing tumour growth in the long term, and is often used as an adjunct to surgery when this has failed to achieve an adequate cure. Although it is proven to reduce the risk of post-operative tumour progression, this is almost invariably at the cost of eventual hypopituitarism (Littley *et al.* 1991), which then requires life-long multiple-hormone replacement therapy. Thus, patients require regular screening for progressive hypopituitarism, and eventually need substitution therapy with corticosteroids, growth hormone, thyroxine and sex steroids (or gonadotrophins to achieve fertility).

In summary, therefore, despite important advances, pituitary tumour therapy at present is often unsatisfactory. Surgery is commonly inadequate to achieve endocrine cure, medical therapies have significant long-term side-effects and expense, and irradiation causes hypopituitarism, necessitating life-long replacement therapy. Therefore, it is timely to consider whether recent advances in pituitary cell and molecular biology may be used to design new therapies, with the aim of selective ablation of tumour cells without damaging the normal pituitary gland.

Molecular biology of the pituitary

In the past 12 years a network of transcription factors has been discovered that determines cell lineage development in the embryonic pituitary (Treier *et al.* 1998), and much of this work has given important insights into how hormone genes are expressed in the mature anterior pituitary gland.

For example, the human prolactin gene is a single copy gene which is expressed in lactotrophic cells of the anterior pituitary gland, and also at lower levels in a number of extra-pituitary tissues. The pituitary-specific expression is an example of cell-type-specific promoter regulation. The human prolactin gene has been studied in detail and contains an extensive upstream flanking region (5000 bp) which is responsible for the pituitary lactotroph specificity of gene expression. This region of the gene comprises multiple binding sites for the pituitary homeodomain transcription factor, Pit-1, and these binding sites are essential to mediate hormonal and intracellular regulation of gene expression (see, for example, Peers *et al.* 1990, Berwaer *et al.* 1991, Hoggard *et al.* 1991, Takasuka *et al.* 1998). Similar studies have been carried out on the GH gene promoter, which also confers pituitary cell-type-specificity on transgene expression *in vivo* (for review see Schaufele 1994).

With a substantial background of knowledge concerning the regulation of pituitary hormone gene expression, there is now a strong case for applying this information to develop new tools for therapy. Specifically, for example, the tight transcriptional regulation of the prolactin gene could, in principle, be exploited to direct expression of a desired transgene to lactotrophic cells only, within the mixed cell population found in the intact pituitary gland. If appropriate expression of a marker gene could be shown to be limited to lactotrophic cells, then a 'suicide' gene could also be expressed in a cell-type-specific manner, and hence ablate lactotrophic cells while leaving other cell types unaffected. Thus, there is now a powerful case for developing a proof of concept using prolactin promoter-directed gene therapy in the context of well-validated cell culture systems and whole animal physiology.

Adenoviruses as tools for gene transfer

Many viral and non-viral delivery systems have been developed for *in vitro* and *in vivo* gene transfer, and since they have been reviewed extensively they will not be the focus of this review (Castro 1999, Morsy & Caskey 1999). We will concentrate on recombinant adenoviruses, since this is the delivery vector which we and others have successfully used for gene transfer into the anterior pituitary gland both *in vitro* (Castro *et al.* 1997, Lee *et al.* 1999, Neill *et al.* 1999) and *in vivo* (Windeatt *et al.* 1999a).

First generation recombinant adenoviruses vectors (RAbs) lack most of the E1A and E1B regions (see below), and are usually propagated in 293 cells which provide the products of the E1A genes *in trans* (reviewed in Morsy & Caskey 1999). The lack of the E1A region renders the recombinant viruses unable to replicate except in this laboratory cell culture system, so that they cannot normally propagate *in vivo* to cause a clinical disease. These vectors have several characteristics which make them very attractive candidates for *in vivo* gene transfer. They are safe, having been used extensively as a vaccine. Wild type adenoviruses cause mostly benign upper respiratory tract infections, and they have not been associated with oncogenicity in humans. Their genome is well characterised and straightforward methods to manipulate it have been described. The recombinant vectors are stable, can be produced to very high titres in the laboratory (10^{11} - 10^{12} pfu/ml), can infect a broad range of both dividing and post-mitotic cell types, and have an insert capacity of up to 8 kbp.

The adenoviral genome is a linear, double stranded DNA molecule of approximately 36 kbp. At each terminus of the genome, there are short inverted terminal repeats (ITRs) which are required for viral DNA replication. The packaging sequence (Ψ) mediates DNA-capsid recognition, and encapsidation. The viral gene products are classified as 'early' (E1-E4) or 'late' (L1-L5), in relation to their expression either before or after the start of viral DNA replication. E1A encodes proteins involved in transcription activation and induction of host cells to enter S phase of the cell cycle. E1B encodes proteins which in conjunction with E1A proteins induce host cell growth. E2 encodes three proteins involved in DNA replication and transcription regulation. E3 encodes proteins which are involved in the modulation of the immune response to adenoviral infections. E4 encodes proteins which regulate transcription and transition from early to late gene expression, shut-off of host cell protein synthesis, transport of mRNA, replication of viral DNA and assembly of the viral particle. The late genes (L1-L5) are involved in the synthesis and assembly of proteins which constitute the viral capsid. The transcription units encoding the gene of choice driven by specific promoters are usually inserted into the E1 or E3 region of the adenoviral genome (Lowenstein *et al.* 1996).

In spite of the E1 deletion within the genome of recombinant adenoviral vectors, there is some expression of downstream viral genes. These viral proteins induce strong immune responses in the host, mediated by cytotoxic T lymphocytes (CTLs). This can, in turn, lead to the elimination of infected host cells within a few weeks of *in vivo* vector delivery in peripheral organs such as the liver. This causes major reductions in transgene expression (Jooss *et al.* 1996). In the brain, the scenario is

more complex, due to the immune-privileged status of the brain parenchyma (Wood *et al.* 1996, Lowenstein *et al.* 1999). Transgene expression after vector delivery in the central nervous system lasts longer, usually months, even in the absence of powerful immune suppressants (Geddes *et al.* 1997). Antibodies produced against viral capsids, viral gene products, or therapeutic gene products (if immunogenic) can also limit re-administration of the vector. It has further been shown that, after adenoviral delivery, very early inflammatory responses and cytokine release occur which are most likely mediated by the viral capsid (Cartmell *et al.* 1999).

Modified RAdS have been generated to reduce their immunogenicity and increase cloning capacity. These include E1-deleted vectors which also carry deletions or temperature-sensitive mutations in the E2 region and/or E4 region (reviewed in Morsy & Caskey 1999). Recently, a helper-dependent adenoviral vector system has been described in which all adenoviral coding regions have been eliminated from the vector. This system is known as a 'gutless' adenovirus vector and should have reduced immunogenicity and large cloning capacity (~30 kbp of foreign DNA; Morsy & Caskey 1999).

Recombinant adenoviruses have been shown to be very good delivery vectors for the anterior pituitary gland *in vitro* (Castro *et al.* 1997, Lee *et al.* 1999, Neill *et al.* 1999) and *in vivo* (Windeatt *et al.* 1999b). It has recently been shown that, using cell-type-specific promoters within RAdS, it is possible to restrict the expression of a marker or therapeutic gene to predetermined cell types (neurones or glial cells) (Shering *et al.* 1997) and also reduce peripheral liver toxicity after systemic administration (Morelli *et al.* 1999). This potential for cell-type-specific restriction of RAd transgene expression has now been proved to work in the pituitary gland, using both the prolactin and GH gene promoters (Lee *et al.* 1999, Windeatt *et al.* 1999a,b). Transgene inducibility utilising the tetracycline-responsive transcriptional system (Gossen *et al.* 1995) has been shown to be functional when inserted into RAdS both *in vitro* (Yoshida & Hamada 1997) and *in vivo* (Harding *et al.* 1998), allowing tight regulation of gene expression. The cell-type specificity has more recently been combined with inducibility by using cell-type-specific promoters driving the tetracycline-inducible system (Smith-Arica *et al.* 1999). Thus, it is now possible to direct expression of a desired transgene within the anterior pituitary gland both *in vitro* and *in vivo*, restricted to specific cell types, and activated by inducing agents for specified periods.

Therapeutic modalities which could be applied to the treatment of pituitary tumours range from conditional cytotoxic approaches (i.e. herpes simplex virus type 1-thymidine kinase (HSV1-TK) followed by ganciclovir treatment; *E. coli* cytosine deaminase which activates 6-fluorocytosine; etc.) to direct cytotoxins (i.e. diphtheria

toxin, pseudomonas exotoxin A), inhibition of growth factors and their receptors which modulate angiogenesis, or expression of tumour suppressor genes or genes that modulate the cell cycle (reviewed in Moolten 1994, Castro 1999). All these therapeutic modalities pose advantages and disadvantages, and they will need to be assessed experimentally *in vitro* and *in vivo* in suitable animal models before their suitability for pituitary tumour treatment can be determined (Lowenstein 1997).

Assessment of efficacy and safety of adenoviral gene therapy in pituitary disease

The anatomy of the pituitary gland, and its highly vascular sinusoid system, may require specific safety considerations to be tested in different animal models. For example, dissemination of adenovirus outwith the pituitary may be important: a recent study of injection into rat medullary thyroid carcinomas showed that intratumorally injected virus could enter the circulation, infect peripheral tissues and express transgenes driven by the human cytomegalovirus promoter. Variable degrees of lymphocytic infiltration into the liver were found histologically, although there was no indication of hepatic dysfunction (Zhang *et al.* 1999); similar responses are likely to occur in the pituitary and their functional consequences will have to be evaluated.

A major consideration in the development of an acceptable adenoviral treatment is the choice of the most appropriate animal model as an intermediary between the *in vitro* cell culture models and widespread applications in clinical trials. Mice have the advantage of easily providing high numbers of experimental animals, and facilitate tissue analysis to assess potential viral dissemination and 'ectopic' transgene expression, given the small size of the organs to be assessed. Furthermore, the immune-compromised nude mouse allows direct assessment of the efficacy of vector treatment on implanted tumours in the absence of an adaptive immune response (Lee *et al.* 1999). However, the major disadvantage of using mice is their small size, which dramatically limits the ability to effectively monitor endocrine changes occurring after any treatment, particularly in the case of changes in the pattern of pulsatile secretion which is characteristic of pituitary hormones. Serial blood sampling is an heroic task in mice, and if achieved, results in very small blood or plasma volumes available for analysis. Hence, only one or two hormones may be analysed at any one time. Furthermore, the small size of the mouse pituitary gland does not allow any assessment of potential spread of injected virus in a realistic model of the human pituitary. Rats are the obvious alternative. Their larger size allows repeated blood sampling with larger sample sizes and easier analysis of hormonal changes. The larger pituitary also allows better

analysis of the spread and distant transgene expression of the virus. Nevertheless, the pituitary is still very small in comparison with the human gland, smaller than most microadenomas, and this limits the investigation of the degree of spread of the gene construct in comparison with the situation faced by the neurosurgeon treating a pituitary tumor.

In sheep, we have an excellent alternative model. Both body and pituitary size are similar to the human and the anatomy of the pituitary is also very similar. Repeated blood samples giving sufficient sample size for multiple hormone measurement allow a complete analysis of changes in the pulsatile secretion pattern of all pituitary hormones over prolonged periods. Thus monitoring the response to induced ablation of pituitary cell types can be carried out effectively. Furthermore, the large size of the pituitary provides an ideal model to determine the effect of changes in dose and volume on the spread of the adenoviral vectors and resultant ability to ablate the cells of choice. It is not clear whether one should use primates to test the efficacy of the treatment before use in man. Marmoset monkeys could be used, but their small body and pituitary size, equivalent to a rat, the difficulty of taking multiple blood samples, and the relative lack of detailed knowledge of their endocrine function, do limit the potential for use at this time. In future as hormone assays are refined for the marmoset, it may become the model of choice. Old world primates are limited in availability for the final experiments required to determine the efficacy of the treatments, but these animals are clearly the closest to man.

In all these animal models, the major drawback is the lack of spontaneous pituitary tumours which could be treated as a true test of the success of ablative therapy. As factors which are associated with pituitary tumours in man are identified, it may become possible to induce tumour development in these animal models, allowing a more direct analysis of the effects of adenoviral therapy. Our initial results in sheep using adenoviral vectors have confirmed promoter-determined cell specificity of expression and have allowed an analysis of the spread of the vector within the pituitary after injection into multiple sites (authors' unpublished data). These results support our contention that the sheep provides an ideal model as the intermediary between cell culture experiments and clinical trials of gene therapy for human pituitary tumours.

Potential practicalities of clinical use – acceptability and ethics of gene therapy for benign disease

Conventional therapies do fail in the day-to-day treatment of pituitary tumours. In many cases, tumours are not completely removed, or they are resistant to combined pharmacotherapy, surgery, and radiation. Could gene

therapy be used in such cases? Gene therapy is already being used for the treatment of incurable brain glioblastoma, and infiltrating pituitary tumours could potentially also be treated. Such non-responsive pituitary tumours are as life-threatening as glioblastoma multiforme. Since, in these cases, the tumours have failed to respond to conventional treatment, and because of their location deep within the brain, they would be suitable for stereotactic delivery of gene therapy vectors for tumour cell killing. Such cases, even though their numbers will possibly be very small, could be treated by gene therapy without raising any new ethical issues.

Could, however, pituitary microadenomas be treated by gene therapy? In this case, we are now considering a tumour which, in a high percentage of cases, responds to conventional therapy. To construct a case for gene therapy of pituitary microadenomas, one would have to argue that gene therapy approaches are truly non-cytotoxic to non-tumoral pituitary cells or systemically, and are potentially as efficient as pharmacotherapy, surgery and radiotherapy. This will be an interesting challenge to meet.

If infection with viral vectors could be made 100% tumour cell-specific, if expression of transgenes could be made conditional on the administration of small molecular weight molecules, and if the general non-specific toxicity could be reduced substantially, then gene therapy could eventually compete with conventional therapies. However, it will be difficult for gene therapy to compete with the conventional therapies which are used regularly to treat the majority of affected patients. Currently, viral vector targeting is very good, but complete and specific targeting of both the virions and the transgenic expression cassettes completely restricted to target cells, remains to be achieved. However, if these objectives are met, gene therapy ought to be considered a viable alternative to other conventional strategies.

What is the role that gene therapy could play in the treatment of benign disease? It is unlikely that it would become acceptable as an alternative treatment to conventional, proven, and clinically effective treatments. A case in point that serves as comparison is the discussion regarding a gene therapy trial for retinoblastoma, recently held at the Office for Recombinant DNA Activities (RAC) of the National Institutes of Health, USA. The aim of the trial was to preserve vision in an eye affected with retinoblastoma. Given that surgical treatment is effective in close to 100% of cases, to preserve vision was regarded as providing an improvement in quality of life, rather than preserving life. RAC was rather critical of the idea of using gene therapy in such a trial, given that a possible improvement in quality of life had to be balanced with a risk that the treatment may delay the use of conventional treatment, thus risking treatment failure and death (Garber 1999).

However, as gene therapy progresses and becomes safer, its application to less life threatening diseases is likely to become more acceptable. Thus, the versatility which gene therapy brings to the pharmacological treatment of disease will be recognised as allowing an improvement in treatment quality and clinical outcome for various diseases. We foresee that this will also be the case for pituitary tumours, where the large number of strategies based on gene therapy could be harnessed to improve the management of patients with pituitary tumours.

References

- Ahmed S, Elsheikh M, Stratton IM, Page RCL, Adams CBT & Wass JAH 1999 Outcome of transphenoidal surgery for acromegaly and its relationship to surgical experience. *Clinical Endocrinology* **50** 561-567.
- Bates AS, van't Hoff W, Jones JM & Clayton RN 1993 An audit of outcome of treatment in acromegaly. *Quarterly Journal of Medicine* **86** 293-299.
- Berwaer M, Monget P, Peers B, Mathy-Hartert M, Bellefroid E, Davis JRE, Belayew A & Martial JA 1991 Multihormonal regulation of the human prolactin gene – expression from 5000 bp of its upstream sequence. *Molecular and Cellular Endocrinology* **80** 53-64.
- Bevan JS, Webster J, Burke CW & Scanlon MF 1992 Dopamine agonists and pituitary tumor shrinkage. *Endocrine Reviews* **13** 220-240.
- Cartmell T, Southgate T, Poole S, Castro MG, Lowenstein PR & Luheshi GN 1999 IL-1 mediates a rapid inflammatory response following adenoviral vector delivery into the brain. *Journal of Neuroscience* **19** 1517-1523.
- Castro MG 1999 Gene therapy strategies for the treatment of pituitary tumours. *Journal of Molecular Endocrinology* **22** 9-18.
- Castro MG, Goya RG, Sosa YE, Rowe J, Larregina A, Morelli A & Lowenstein PR 1997 Expression of transgenes in normal and neoplastic anterior pituitary cells using recombinant adenoviruses: long term expression, cell cycle dependency, and effects on hormone secretion. *Endocrinology* **138** 2184-2194.
- Davis JRE, Sheppard MC & Heath DA 1990 Giant invasive prolactinoma – a case report and review of nine further cases. *Quarterly Journal of Medicine* **74** 227-238.
- Garber K 1999 RAC urges changes to retinoblastoma plan. *Science* **284** 2066.
- Geddes BJ, Harding TC, Lightman SL & Uney JB 1997 Long term gene therapy in the CNS: reversal of hypothalamic diabetes insipidus in the Brattleboro rat by using an adenovirus expressing arginine vasopressin. *Nature Medicine* **3** 1402-1404.
- Gossen M, Freundlieb S, Bendre G, Muller G, Hillen W & Bujard H 1995 Transcriptional activation by tetracyclines in mammalian cells. *Science* **68** 1766-1769.
- Harding TC, Geddes BJ, Murphy D, Knight D & Uney JB 1998 Switching transgene expression in the brain using an adenoviral tetracycline-regulatable system. *Nature Biotechnology* **16** 553-555.
- Hoggard N, Davis JRE, Berwaer M, Monget P, Peers B, Belayew A & Martial JA 1991 Pit-1 binding sequences permit calcium regulation of human prolactin gene expression. *Molecular Endocrinology* **5** 1748-1754.
- Jooss K, Ertle HCJ & Wilson JM 1996 Cyclophosphamide diminishes inflammation and prolongs transgene expression following delivery of adenoviral vectors to mouse liver and lung. *Human Gene Therapy* **7** 1555-1566.
- Lee EJ, Anderson LM, Thimmapaya B & Jameson JL 1999 Targeted expression of toxic genes directed by pituitary hormone promoters: a potential strategy for adenovirus-mediated gene therapy of pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* **84** 786-794.
- Lissett CA, Peacey SR, Laing I, Tetlow L, Davis JRE & Shalet SM 1998 The outcome of surgery for acromegaly: the need for a specialist pituitary surgeon for all types of growth hormone-secreting adenomas. *Clinical Endocrinology* **49** 653-657.
- Little MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G & Sutton ML 1991 Hypopituitarism following external radiotherapy for pituitary tumours in adults. *Quarterly Journal of Medicine* **70** 145-160.
- Lowenstein PR 1997 Why are we doing so much cancer gene therapy? Disentangling the scientific basis from the origins of gene therapy. *Gene Therapy* **4** 755-756.
- Lowenstein PR, Shering AF, Bain D, Castro MG & Wilkinson GWG 1996 The use of adenovirus vectors to transfer genes to identified target brain cells *in vitro*. In *Gene Transfer into Postmitotic Neurons: Towards Gene Therapy of Human Neurological Disorders*, pp 93-114. Eds PR Lowenstein & LW Enquist. Chichester: John Wiley and Sons.
- Lowenstein PR, Southgate T, Smith-Arica J, Smith J & Castro MG 1998 Gene therapy for neurological disorders: towards therapeutic intervention in the Lesch-Nyhan syndrome. In *Progress in Brain Research, Neuronal Degeneration and Regeneration: from Basic Mechanisms to Prospects for Therapy*, pp 485-501. Eds FW van Leuwen & J Verhaagen. Amsterdam: Elsevier Science.
- Lowenstein PR, Thomas CE & Castro MG 1999 Politically correct gene therapy? A 'clean environment' improves gene delivery to the brain! *Gene Therapy* **6** 463-464.
- Miyai K, Ichihara K, Kondo K & Mori S 1986 Asymptomatic hyperprolactinaemia and prolactinoma in the general population - mass screening by paired assays of serum prolactin. *Clinical Endocrinology* **25** 549-554.
- Molitch ME, Thorner MO & Wilson C 1997 Management of prolactinomas. *Journal of Clinical Endocrinology and Metabolism* **82** 996-997.
- Moolten FL 1994 Drug sensitivity ('suicide') genes for selective cancer control strategy. *Cancer Gene Therapy* **1** 279-287.
- Morelli A, Larregina A, Smith J, Dewey R, Southgate T, Fontana A, Castro MG & Lowenstein PR 1999 Reduced systemic toxicity of recombinant adenovirus vectors expressing the apoptotic molecule Fas-L driven by cell-type specific promoters. *Journal of General Virology* **80** 571-583.

- Morsy MA & Caskey CT 1999 Expanded-capacity adenoviral vectors: the helper dependent vectors. *Molecular Medicine Today* **5** 18-24.
- Neill JD, Lois C, Musgrove L, Duck W & Sellers JC 1999 High efficiency method for gene transfer in normal pituitary gonadotropes: adenoviral-mediated expression of G protein-coupled receptor kinase 2 suppresses luteinizing hormone secretion. *Endocrinology* **140** 2562-2569.
- Orme SM, McNally RJQ, Cartwright RA & Belchetz PE 1998 Mortality and cancer incidence in acromegaly: a retrospective cohort study. *Journal of Clinical Endocrinology and Metabolism* **83** 2730-2734.
- Peers B, Voz ML, Monget P, Mathy-Hartert M, Berwaer M, Belayew A & Martial JA 1990 Regulatory elements controlling the expression of the human prolactin gene. *Molecular and Cellular Biology* **10** 4690-4700.
- Schafele F 1994 Regulation of expression of the growth hormone and prolactin genes. In *The Pituitary Gland*, edn 2, pp 91-116. Ed H Imura. New York: Raven Press.
- Sheppard MC 1994 Aims of treatment and definition of cure. In *Treating Acromegaly – 100 Years On*, pp 17-31. Ed JAH Wass. Bristol: Society for Endocrinology.
- Shering AF, Bain D, Stewart K, Epstein AL, Castro MG, Wilkinson GWG & Lowenstein PR 1997 Cell type-specific expression in brain cell cultures from a short human cytomegalovirus major immediate early promoter depends on whether it is inserted into herpes virus or adenovirus vectors. *Journal of General Virology* **78** 445-459.
- Smith-Arica J, Smith J, Davis JRE, Lowenstein PR & Castro MG 1999 CNS specific and inducible gene expression from first generation adenoviral vectors *in vitro*: possibilities for gene therapy. *British Neuroscience Association (Abstract)* **15** 57.
- Soule SG, Conway GS & Jacobs HS 1996 The outcome of hypophysectomy for prolactinomas in the era of dopamine agonist therapy. *Clinical Endocrinology* **44** 711-716.
- Takasuka N, White MRH, Wood CD, Robertson WR & Davis JRE 1998 Dynamic changes in prolactin promoter activation in individual living lactotrophic cells. *Endocrinology* **139** 1361-1368.
- Thomson JA, Davies DL, McLaren EH & Teasdale GM 1994 10-Year follow-up of prolactinoma treated by transphenoidal surgery. *British Medical Journal* **309** 1409-1410.
- Treier M, Gleiberman AS, O'Connell SM, Szeto DP, McMahon JA, McMahon AP & Rosenfeld MG 1998 Multistep signaling requirements for pituitary organogenesis *in vivo*. *Genes and Development* **12** 1691-1704.
- Webster J, Piscitelli G, Polli A, Ferrari CI, Ismail I & Scanlon MF 1994 A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. *New England Journal of Medicine* **331** 904-909.
- Windeatt S, Perone M, Dewey R, Southgate A, Larregina A, Morris I, Lowenstein PR & Castro MG 1999a Gene therapy using herpes simplex type 1 thymidine kinase (HSV1-TK) for the treatment of prolactin-secreting adenomas. *Journal of Endocrinology* **160** P152.
- Windeatt S, Perone M, Southgate T, Davis JRE, Lowenstein PR & Castro MG 1999b Development of a cell-type-specific recombinant adenovirus for transgene expression in pituitary lactotrophic cells. *Journal of Endocrinology* **160** P156.
- Wood MJA, Charlton HM, Wood KJ, Kajiwarra K & Byrnes AP 1996 Immune responses to adenovirus vectors in the nervous system. *Trends in Neurosciences* **19** 497-501.
- Yoshida Y & Hamada H 1997 Adenovirus-mediated inducible gene expression through tetracycline-controllable transactivator with nuclear localization signal. *Biochemical and Biophysical Research Communications* **230** 426-430.
- Zhang R, Straus FH & deGroot LJ 1999 Effective genetic therapy of established medullary thyroid carcinomas with murine interleukin-2: dissemination and cytotoxicity studies in a rat tumor model. *Endocrinology* **140** 2152-2158.