Gene Therapy for Pituitary Tumors

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Abstract

Pituitary tumors are the most common primary intracranial neoplasms. Although most pituitary tumors are considered typically benign, others can cause severe and progressive disease. The principal aims of pituitary tumor treatment are the elimination or reduction of the tumor mass, normalization of hormone secretion and preservation of remaining pituitary function. In spite of major advances in the therapy of pituitary tumors, for some of the most difficult tumors, current therapies that include medical, surgical and radiotherapeutic methods are often unsatisfactory and there is a need to develop new treatment strategies. Gene therapy, which uses nucleic acids as drugs, has emerged as an attractive therapeutic option for the treatment of pituitary tumors that do not respond to classical treatment strategies if the patients become intolerant to the therapy. The development of animal models for pituitary tumors and hormone hypersecretion has proven to be critical for the implementation of novel treatment strategies and gene therapy approaches. Preclinical trials using several gene therapy approaches for the treatment of anterior pituitary diseases have been successfully implemented. Several issues need to be addressed before clinical implementation becomes a reality, including the development of more effective and safer viral vectors, uncovering novel therapeutic targets and development of targeted expression of therapeutic transgenes. With the development of efficient gene delivery vectors allowing long-term transgene expression with minimal toxicity, gene therapy will become one of the most promising approaches for treating pituitary adenomas.

Keywords

Pituitary tumorigenesis; viral vectors; prolactinoma; somatotroph adenoma; Cushing's syndrome

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INTRODUCTION

Pituitary tumors are very common. Most of them show slow growing over many years and are considered typically benign. While the majority of pituitary tumors remain clinically silent, others can cause severe and progressive disease. They may compromise critical surrounding brain structures and/or cause manifestations due to altered secretion of hormones. Considering the high incidence of pituitary tumors and their potential induction of endocrine and neurological disorders, the improved diagnosis and treatment of pituitary adenomas should

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have important benefits. The aim of therapy of pituitary tumors is the elimination or reduction of the tumor mass, thus preventing damage of surrounding structures, normalization of hormone secretion and preservation of remaining pituitary function. Current therapeutic options for pituitary tumors include medical, surgical and radiotherapeutic methods. Although major advances have been made in the therapy of pituitary tumors, for some of the most difficult tumors, only partial success has been possible. Also, in some cases, patients do not tolerate medical treatment and/or the tumors do not respond to current therapy, they recur or become invasive. Therefore, pituitary tumor therapy is often unsatisfactory and there is a need to develop new treatment strategies. Gene therapy, the use of nucleic acids as drugs, has emerged as a promising therapeutic option for the treatment of pituitary tumors. Expression of transgenes whithin the anterior pituitary can be modulated by small molecules, such as tetracycline analogues, in a cell-type specific and temporal fashion making possible to develop gene therapy strategies to human pituitary adenomas and more aggressive tumors affecting the pituitary gland.

EPIDEMIOLOGY OF PITUITARY TUMORS

Pituitary tumors are the most common primary intracranial neoplasms. Several studies show that pituitary adenomas account for 6.6–9.1% of all brain and central nervous system tumors, occurring at an incidence rate between 0.8–8 per 100,000 person/years [Nilsson *et al.*, 2000; Robinson *et al.*, 1979; Surawicz *et al.*, 1999], with a peak incidence that occurs earlier in women (20–45 years) than in men (35–60 years) [Davis *et al.*, 2001a]. The development of these tumors are infrequent during childhood [Davis *et al.*, 2001a; Kane *et al.*, 1994; Mukai *et al.*, 1986].

In a metaanalysis conducted by Ezzat *et al.*, [Ezzat *et al.*, 2004] they found an overall estimated prevalence of pituitary adenomas of 16.7% (14.4% in autopsy studies and 22.5% in non-invasive imaging studies). In addition, magnetic resonance imaging (MRI) has shown lesions suggestive of pituitary adenomas in 10% of normal female volunteer subjects [Swensen *et al.*, 2002].

Although most studies indicate a similar sex incidence of pituitary tumors, a female predominance was reported [Gold, 1981; Percy *et al.*, 1972].

CLASSIFICATION AND CLINICAL ASPECTS OF PITUITARY TUMORS

The majority of pituitary tumors are non-metastatic pituitary adenomas that remain either within the sella or may exhibit expansive growth to surrounding tissue. These invasive tumors are not considered to be malignant, even when dural invasion may be marked. True carcinomas are defined only by the presence of craniospinal and/or systemic metastases and are considered to be rare, with an incidence of less than 0.5% of symptomatic pituitary tumors [Kaltsas *et al.*, 2005].

Pituitary tumors are classified by their size and hormonal activity. Considering their size, pituitary adenomas are divided into microadenomas (<10 mm) with no changes in the sella (grade 0) or minor changes in the sella (grade 1), and macroadenomas (>10 mm) with diffuse enlargement of the sella (grade 2) or with focal (grade 3) or extensive destruction of the sella (grade 4) [Swensen *et al.*, 2002]. Macroadenomas come to medical attention through pressure effects such as headache or progressive visual failure, whereas microadenomas are usually identified during investigation of a clinical endocrine syndrome, or may be discovered as incidental findings during brain scanning for other causes.

Pituitary adenomas are classified according to the characteristic hormone staining, their patterns of hormone production and their clinical presentation. Pituitary tumors are described as functioning if they over-produce any of the anterior pituitary hormone (prolactin, GH,

ACTH, FSH, LH or TSH), or non-functioning if no clinical syndrome is apparent, although some of these tumors produce gonadotropin/TSH alpha subunit (Table 1).

Prolactinoma (lactotroph tumor) is the most frequent pituitary tumor subtype but, if surgical samples are examined, this subtype would be much less prevalent due to the primary treatment of prolactinomas with dopamine agonist drugs [Davis *et al.*, 2001a]. Immunohistochemical staining revealed prolactin-producing cells in 25–41% of tumor specimens [Ezzat *et al.*, 2004]. It has been reported that 35% of pituitary tumors in women produce prolactin and an additional 6% produce prolactin together with other pituitary hormones [Swensen *et al.*, 2002]. Excessive prolactin secretion induces oligomenorrhea or amenorrhea and galactorrhea and, in general, prolactinomas come evident in women because these signs. Higher incidence of prolactinomas in women may result from early presentation of symptoms of hyperprolactinemia such as galactorrhea [Monson, 2000]. In men, lactation is very rare and symptoms of hypogonadism such as impotence and lack of libido tend to come to medical attention relatively late. Although there may also be sex differences in tumor behaviour, the delayed diagnosis may explain why prolactinomas in men are larger than in women [Davis *et al.*, 2001a].

Growth hormone-secreting pituitary adenomas account for 10–20% of pituitary tumors and cause the clinical syndrome of acromegaly. Acromegaly has a higher prevalence in women [Bengtsson *et al.*, 1988; Etxabe *et al.*, 1993; Monson, 2000]. More than 98% of patients with acromegaly have GH-secreting pituitary adenomas [Arafah *et al.*, 2001]. The GH excess results in overgrowth of many tissues through the induction of high levels of insulin-like growth factor I (IGF-I). The changes in the growth rate of soft tissues occur slowly and the diagnosis is often delayed for many years. Therefore these tumors are commonly large by the time they are diagnosed, and may also give mass effects such as visual impairment [Davis *et al.*, 2001a]. Approximately 12% of pituitary tumors in women produce GH, and an additional 6% produce GH with prolactin [Swensen *et al.*, 2002]. In fact, about 30% of patients with GH-secreting pituitary adenomas have hyperprolactinemia as a result of co-secretion of GH and prolactin. However, the rise in circulating levels of prolactin may be secondary to compression of the portal vessels by a mass effect of the tumor [Arafah *et al.*, 2001].

Corticotroph tumors secreting ACTH and other proopiomelanocortin-derived peptides cause the clinical syndrome of Cushing's disease with ACTH-dependent adrenal hyperplasia and high circulating levels of glucocorticoids. Cushing's syndrome refers to a state of hypercortisolism regardless of its etiology. However, Cushing's disease is a state of hypercortisolism caused by excess pituitary secretion of ACTH. Corticotroph tumors represent approximately 5–15% of all pituitary adenomas and are seen predominantly in women. [McComb *et al.*, 1983; Monson, 2000; Swensen *et al.*, 2002]. About 90% of ACTH-secreting pituitary tumors are microadenomas [Shimon *et al.*, 1998]. In fact, it has been reported that MRI scan do not detect demonstrable tumors in approximately 40% of patients with active Cushing disease [Watson *et al.*, 1998]. Almost all patients with Cushing's disease have an associated ACTH-secreting pituitary adenoma that is the most common cause of endogenous hypercortisolism, accounting for approximately 65–70% of all cases of Cushing's syndrome [Arafah *et al.*, 2001]. The chronic excess in the endogenous production of cortisol results in obesity, diabetes mellitus, hypertension, osteoporosis, and mood changes including depression, mania, anxiety disorders, and cognitive dysfunction.

Gonadotroph adenomas account for 10–15% of all pituitary adenomas. In women, about 8% of pituitary adenomas produce gonadotropins [Arafah *et al.*, 2001]. Pituitary tumors secreting FSH or LH are often classified as nonfunctioning adenomas, although menstrual cycle disorders are common [Swensen *et al.*, 2002].

Thyrotroph adenomas are rare, representing 0.5–2% of pituitary neoplasms. More than 70% are macroadenomas [Beck-Peccoz *et al.*, 1996]. The incidence is higher in females than in males [Arafah *et al.*, 2001; Swensen *et al.*, 2002]. Symptoms of hyperthyroidism such as tachycardia, weight loss, heat intolerance, and cardiac dysrhythmias are identical to hyperthyroidism due to thyroid disease but, instead of absent serum TSH, patients have paradoxically elevated serum TSH levels [Swensen *et al.*, 2002]. Some of these tumors stain positively for GH, in addition to TSH, despite the absence of any clinical signs of excessive GH secretion [Arafah *et al.*, 2001].

The second most common cause of pituitary tumors, representing about 30% of all pituitary adenomas, are nonfunctioning adenomas, and are the most common subtype of macroadenomas. As it has been stated above, many of these non-functioning tumors express gonadotropin subunits, but without evident clinical signs. However, 5–10% of all pituitary tumors are truly non-functional and are referred to as null-cell adenomas. These tumors are frequently diagnosed by their mechanical effects (e.g. headaches, visual symptoms) and variable degrees of hypopituitarism [Arafah *et al.*, 2001]. The cause of hypopituitarism was a pituitary tumor in 61%, a non-pituitary tumor in 9% and a non-tumor cause in 30% [Regal *et al.*, 2001].

Pituitary adenomas are associated with many known cancer syndromes, including MEN-1. However, only a 3% of pituitary tumors are associated with this or other familial syndromes [Davis *et al.*, 2001a]. Prolactinoma is the most common pituitary tumor associated with MEN-1, but somatotroph tumors are more frequent in patients above 40 years old [Monson, 2000].

MECHANISMS OF PITUITARY TUMORIGENESIS

Pituitary adenomas subjected to surgery are predominantly monoclonal in origin and roughly half are aneuploid, indicating genetic instability [Alexander *et al.*, 1990; Herman *et al.*, 1990; Levy *et al.*, 2003]. Few are associated with the classical mechanisms of tumor formation, and mutations in classic oncogenes and tumor suppressor genes are not frequently observed in pituitary tumors [Asa *et al.*, 2002]. A small percentage of pituitary tumors, mostly prolactinomas, are associated with the multiple endocrine neoplasia type I syndrome, an inherited disorder [Melmed, 2003]. Also, Protein kinase A regulatory subunit 1 (RPKA1) germ-line mutations have been identified in patients with Carney's Complex, an autosomal dominant disorder that predisposes to pituitary tumors [Lania *et al.*, 2004].

Mutations in cell-cycle genes have been associated with pituitary tumors and therefore are possible targets for gene therapy. Loss of function of growth suppressor genes such as Rb, p16, p27 and GaDD46γ may be involved in pituitary tumorigenesis [Asa *et al.*, 2002; Melmed, 2003; Musat *et al.*, 2004]. The tumor suppressor gene p53 mutations have been observed in almost all pituitary carcinomas but not in benign adenomas [Kaltsas *et al.*, 2005; Melmed, 2003; Saeger, 2004]. Overexpression of Pituitary tumor transforming gene (PTTG), an oncogene expressed abundantly in most tumors, induces cellular transformation. PTTG has been implicated in various cellular processes including sister chromatid separation during cell division as well as induction of apoptosis through p53-dependent and p53-independent mechanisms [Hamid *et al.*, 2004; Yu *et al.*, 2004]. Cells with reduced expression of Pituitary tumor apoptosis gene (PTAG) show a blunted apoptotic response that may underlie oncogenic transformation in the pituitary [Bahar *et al.*, 2004]. Also, changes in the expression of members of the bcl-2 family such as bcl-2 and bax have been suggested to be involved in pituitary tumorigenesis [Gruszka *et al.*, 2004; Ozer *et al.*, 2003; Sambaziotis *et al.* 2003; Turner *et al.*, 2000].

The majority of pituitary tumors bear quantitative rather than qualitative differences in molecular expression compared to the normal pituitary. Tumorigenesis is promoted by hormones and growth factors involved in pituitary development and/or regulation of pituitary function. Although these hormones and growth factors are not likely to be the cause of pituitary tumors, dysregulation of the signalling mechanisms that mediate their pituitary hormone and proliferation responses can promote tumor development. Mutations with gain of function were observed in genes encoding G protein-coupled receptors and signal-transduction enzymes [Lania *et al.*, 2003].

In response to hypothalamic, peripheral and intrapituitary signals, the pituitary gland undergoes reversible changes in cell proliferation leading to hyperplasia or adenoma formation. Overstimulation by hypothalamic hormones such as GHRH might promote cell proliferation in anterior pituitary cells that are already transformed or GHRH-induced hyperplasia provides an adequate environment for cell transformation. Also, TRH overproduction might promote thyrotrope and lactotrope hyperplasia and could lead to adenoma formation [Asa *et al.*, 2002; Thapar *et al.*, 1997]. Pituitary function is also regulated by inhibitory hormones and neurotransmitters. Hypothalamic dopamine tonically inhibits prolactin synthesis through D2 receptors and decreased dopamine inhibition has been proposed to be involved in prolactinoma pathogenesis [Ezzat, 2001]. In fact, some prolactin and somatotropin-secreting tumors bear altered D2 receptors, most of them showing also inactivation of nerve growth factor (NGF) receptors [Fiorentini *et al.*, 2002]. Somatostatin is a hypothalamic hormone that inhibits GH secretion and its expression is reduced in large GH-secreting tumors [Hofland *et al.*, 2004]. Loss of feedback suppression of glucocorticoids, thyroid hormones, gonadal steroids and activins might be a mechanism that is involved in pathological pituitary secretion and proliferation [Asa *et al.*, 2002].

Many of the pituitary-derived growth factors and cytokines are potentially oncogenic [Ray *et al.*, 1997]. Among growth factors, Epidermal growth factor (EGF) and its receptor are expressed in several types of pituitary tumors especially in corticotropinomas [Theodoropoulou *et al.*, 2004]. Overexpression of Transforming growth factor-alpha and loss of Nerve growth factor (NGF) production appear to be involved in the development and progression of prolactin-secreting tumors [Missale *et al.*, 1999]. Several members of the Transforming growth factor-beta and Fibroblast growth factor families as well as Bone morphogenetic factors have been suggested to be involved in pituitary tumorigenesis [Goya *et al.*, 2004; Hentges *et al.*, 2001; Renner *et al.*, 2004]. Among cytokines, interleukin (IL)-6 and leukaemia inhibitory factor (LIF), members of the gp130 cytokine family are also candidates for tumor development or progression [Hanisch *et al.*, 2000; Renner *et al.*, 2004; Yano *et al.*, 1998].

An integrated approach incorporates both the hormone stimulation theory and the intrinsic defect theory of pituitary tumorigenesis [Asa *et al.*, 2002]. Supporting the theory of intrinsic defects are the monoclonal nature of pituitary adenomas [Alexander *et al.*, 1990; Herman *et al.*, 1990; Levy *et al.*, 2003], the low rate of recurrency of pituitary tumors after surgical resection, the absence of hyperplasia of surrounding tissue in most of them and the absence of receptors for hypothalamic hypophysiotropic factors in some pituitary adenomas. Human pituitary adenomas may arise from genetic mutations that alter cell proliferation or survival thus allowing cells to become more responsive to hormones or growth factors.

CURRENT TREATMENT FOR PITUITARY TUMORS

Treatment for pituitary tumors are intended to point endocrine malfunction, i.e., compressive pituitary failure and/or hormone hypersecretion and the effects of central mass. Treatment of pituitary tumors generally involves combination of medical therapy, surgery and radiotherapy

[Kreutzer *et al.*, 2004]. Symptoms and signs of pituitary failure such as hypogonadism, thyroid or adrenal failure are managed by replacement of the deficient hormone. Central mass effects are treated with medical therapy that shrinks or reduces the tumoral mass. Hormone hypersecretion is controlled or abolished by surgery, pharmacological approaches and radiotherapy.

Transphenoidal surgery is highly effective for most pituitary tumors, especially for well circumscribed microadenomas. However, even in experienced hands, surgical resection can be followed by complications such as hypopituitarism or permanent diabetes insipidus. In the case of macroadenomas there is greater risk to damage related structures and the incidence of diabetes insipida is higher. Other complications are CSF leak, meningitis, pneumocephalus, visual deterioration and haematoma [Sudhakar *et al.*, 2004]. Although surgery remains a first choice for treatment of most pituitary tumors except microprolactinoma, its long-term results are often disappointing. It has been reported that in Cushing's disease that underwent transphenoidal microsurgery for ACTH-secreting adenoma, postoperative remission was not achieved in almost 20% of patients. [Hammer *et al.*, 2004].

Hormone secretion from pituitary tumors is associated with the specific phenotipic features and can vary from normal range to excessive levels. However, hormone release from tumor cells can be usually regulated by the same control mechanisms that take effect on normal cells. Dopamine agonist therapy is the treatment of choice for microprolactinomas. Dopamine induces an anti-proliferative effect and cell death via the dopamine D2 receptors [An *et al.*, 2003]. Dopamine agonists such as bromocriptine, quinagolide or cabergoline have been successfully used for the treatment of prolactin-secreting tumors, shrinking the tumoral mass and reducing prolactin hypresecretion [Serri *et al.*, 2003]. However, a considerable rate of side effects are present in long-term treatment with these drugs. Somatostatin analogs, such as octeotride and lanreotide are used to control GH hypersecretion but they often fail to shrink somatotrope tumors. Somatostatin analogs can also reduce TSH secretion and, in some cases, the size of TSH-secreting adenomas. The GH-receptor antagonist pegvisomat may be useful in normalizing IGF-I levels but again they unlikely reduce the tumoral mass.

Radiotherapy has been extensively used for prevention of tumor regrowth after surgery. It has been also a therapeutic option for patients which cannot undergo a surgical procedure or when surgical excision is not viable. A frequent consequence of radiotherapy is the associated hypopituitarism which then requires permanent multiple-hormone replacement therapy. Focused radiotherapy, either in the form of stereotactic multiarc radiotherapy or Gamma-knife that can be applied to a small area, have the advantage to reduce damage to normal pituitary tissue and surrounding vascular and neuronal structures [Kaltsas *et al.*, 2005; Laws *et al.*, 2004].

Major advances have been achieved in the therapy of pituitary tumors over recent years, but despite this their treatment often remains unsatisfactory. In the majority of cases, the treatments currently employed are not sufficiently effective in providing complete cure. Some patients fail to respond to pharmacological therapy or do not tolerate associated side effects.

VIRAL VECTORS FOR GENE THERAPY

Viral vectors are a great tool for delivering therapeutic genes during gene therapy and to date there are a variety of viral vectors to choose from (Table 2). Several viruses have been developed to further the field of gene therapy although there are advantages and disadvantages to most of them. There are primarily two classifications of viral vectors: integrating and nonintegrating. Integrating viruses insert their DNA, along with the therapeutic gene of interest, into the host DNA while the nonintegrating viral DNA remains episomal. Retroviruses, such as MMLV, and lentiviruses, such as HIV, are integrating viruses. The nonintegrating viruses

include adenoviruses, herpesvirus and the adeno-associated viruses (AAV). AAV are sometimes able to integrate but usually do not [Nakai.*et al.*, 2001;Nakai.*et al.*, 2003].

Retroviruses and lentiviruses are RNA viruses which are considered advantageous because they are integrating viruses. The ability to incorporate their DNA into the host allows for the therapeutic gene to be perpetually present in the genome of the cells infected and can be passed on to future generations of that cell. Lentiviruses have an even better advantage due to their ability to transduce dividing and nondividing cells [Blesch, 2004; Carlotti *et al.*, 2004; Jakobsson *et al.*, 2003; Trobridge *et al.*, 2004; Vogel *et al.*, 2004]. This unique characteristic makes them strong candidates for gene therapy. Unfortunately there are disadvantages that go along with these integrating vectors. The possibility of insertional mutagenesis is a big concern or integration within an oncogene which could therefore lead to cancer [Li *et al.*, 2002; Thomas *et al.*, 2003]. With lentiviruses, the fear that recombination could lead to HIV in gene therapy patients is a drawback, even though the vectors have been constructed in such a way that recombining is a miniscule possibility.

As for the non-integrating viral vectors, the adenovirus is a good candidate for gene therapy. This double stranded DNA virus infects cells and its genome enters the nucleus of the cell and remains episomal [Leopold *et al.*, 1998]. The adenoviruses have been made replication defective by deleting the E1 and E3 regions, therefore not only decreasing the possibility of recombinants but also adding the ability to host a larger transgene. Unfortunately, the disadvantage for adenoviruses is that most people have been pre-exposed to an adenovirus and therefore have immunity to the viral proteins. This anti-adenoviral immunity can reduce transgene expression [Thomas *et al.*, 2001] leading to an ineffective therapy. In order to avoid the adverse immune-mediated side effects due to pre-existing immunity, high-capacity, gutless adenoviral vectors have been developed [Lowenstein *et al.*, 2002; Ng *et al.*, 2001; Parks *et al.*, 1996; Umana *et al.*, 2001]. These vectors do not encode, and therefore do not express most of the viral proteins and therefore are much less immunogenic than the first generation adenoviruses [Maione *et al.*, 2001; Schiedner *et al.*, 1998; Thomas *et al.*, 2000; Thomas *et al.*, 2001; Toietta *et al.*, 2003]. Of course, this vector, too, has its limitations. The primary disadvantages with the gutless vector are the difficulty involved in producing large quantities and the possibility of helper virus contamination.

Another commonly used viral vector is the adenoassociated virus (AAV) which is a single stranded DNA virus. The AAV vectors are considered non-integrating vectors although they can sometimes integrate into the host genome within active genes [Nakai *et al.*, 2001; Nakai *et al.*, 2003]. The initial AAV vectors were produced simply by replacing viral genes with the transgene cassette. With recombination being a strong possibility with AAV, future AAV vectors were developed to prevent wild-type AAV from occurring [Samulski *et al.*, 1989]. The downside to AAV vectors is the small packaging capacity (~4–5kb). This has been improved due to the fact that these vectors concatamerize after transduction [Nakai *et al.*, 2000], therefore giving one the ability to put half of a transgene in one vector and half of the transgene in another vector which will end up as a reconstituted functional transgene.

Herpes Simplex Virus type I (HSV) is also a frequent choice for a non-integrating gene therapy vector. This double stranded DNA molecule is deleted of its many (~80) nonessential genes to allow for a larger transgene capacity, approximately 50kb [Glorioso *et al.*, 1995]. Deletion of the immediate early genes, which are involved in lytic infection, allows for a recombination deficient vector. The main disadvantage presented by the HSV vectors is the immune response associated with this virus due to the fact that a large majority of the population has been infected with HSV at some point in their lives [Herrlinger *et al.*, 1998]. These vectors have also been associated with cytotoxicity.

There are several viral vectors to choose from when pursuing gene therapy. All of the vectors have their strong points and their pitfalls (Table 3). The potential of gene therapy is mostly due to the wide variety of vectors and the ability to choose the appropriate vector for each particular target. This exciting new therapeutic field gets closer to clinical success with each step towards advancing the viral vectors in efficiency, cloning capacity, long term gene expression and increased biosafety.

There are many viral vectors used for gene therapy and the above mentioned vectors are promising tools for anterior pituitary therapy. As far as the retroviruses are considered, the lentivirus is a strong candidate and shows great potential for future anterior pituitary gene therapy applications. The main downfall to these viruses is the safety concern which is constantly under scrutiny although much research has gone into making these viruses safe. First generation adenoviruses are a great vector choice but the pre-existing adenoviral immune response poses a serious issue. Therefore, the gutless adenovirus vectors are a much better choice for anterior pituitary gene therapies. The biggest concern with the gutless adenoviral vectors is the possibility of helper virus contamination. A scalable protocol for producing these viruses without significant helper contamination has recently been developed [Palmer and Ng, 2003], therefore it is expected that they will be more widely used for human applications. Another strong vector is AAV but again the immune response can contribute to loss of transgene expression which obviously is a negative factor for this virus. AAVs also have a small cloning capacity and therefore can only be used for a small number of gene therapy approaches. Lastly, the HSV vectors, which have shown promise, tend to be toxic. Overall, the scientific community has developed a wide range of vectors that are suitable for anterior pituitary gene therapy but the continued development of these vectors is necessary.

MODELS FOR PITUITARY ADENOMAS AND HORMONE HYPERSECRETION

The development of animal models for pituitary tumors and hormone hypersecretion has proven to be critical for the implementation of novel treatment strategies and gene therapy approaches. *In vivo* manipulations of animals have enabled the elucidation of the molecular and biochemical pathways involved in pituitary tumorigenesis. The rapid growth of molecular techniques, such as transgenic technology, siRNA, knockout mouse and the establishment of stable anterior pituitary cell lines are crucial tools in the development and testing of novel gene therapies for pituitary tumors, or to control hormone hypersecretion.

The knowledge surrounding human cancers has been highly enriched by the observations made from numerous transgenic mouse model studies [Ying *et al.*, 2003]. Selecting the appropriate experimental model is critical and the value of the model depends on its validity, selectivity, predictability, and reproducibility. In general, animal tumor models can be divided into either spontaneous or transplanted tumor systems. The most common animal species employed for studies related to pituitary tumor genesis are rats and mice. However, the sheep has also proven to be a very useful *in vivo* model to study pituitary physiology and pathogenesis. A recent study demonstrated cell specific expression of a marker gene in sheep after stereotactic injection of adenoviral vectors [Davis *et al.*, 2001b]. This experiment was designed to establish a larger animal model with an anterior pituitary comparable in structure and size to the human gland. In this experiment, the prolactin promoter driving β-galactosidase achieved cell specific expression and maintained normal endocrine function for a short period of time. Another non-rodent animal study involved the monkey pituitary gland and evaluated the mechanisms involved in the normal development of the pituitary gland [Schanke *et al.*, 1997]. These large animal models are useful to develop gene trasfer technologies, optimise viral vector doses to achieve adecuate levels of transgene expression with minimal inflammation or adverse immune reactions, and importantly preserving normal anterior pituitary hormones' secretion. Below we discuss a number of animal models developed over the years and their uses in the

development of novel treatment strategies for pituitary adenomas, pituitary hyperplasia and hormone hypersecretion.

Prolactinomas

The animal models employed to study prolactin-secreting adenomas include three major types: hormonally stimulated, implantation of established prolactin secreting tumor cell lines and sporadic adenomas. Established prolactin secreting cell lines in culture can also be used to develop treatment strategies aimed at inhibiting hormone secretion and cell proliferation.

Estrogen, a powerful steroid hormone, stimulates proliferation of the prolactin-producing lactotroph cells inducing the development of lactotroph hyperplasia in several rat strains [Spady et al., 1999]. The Fisher (344) rat is considered to be the most sensitive strain of rats to estrogen-induced prolactinoma formation. Nonetheless, chronic estrogen treatment also induces the formation of pituitary prolactin-secreting adenomas in male Sprague-Dawley rats. Following estrogen treatment, prolactinomas develop in the adenohypophysis *in situ* and/or the ectopic pituitary gland grafted under the renal capsule in Sprague-Dawley rats [Xu et al., 2000]. A range of estrogen–related compounds such as subcutaneous diethylbestrol or estrone implants and injections of 17β-estradiol were shown to induce lactotroph hyperplasia. [Treip, 1983]. Aged female and male Sprague Dawley and Wistar strain rats have been shown to develop prolactin-secreting pituitary adenomas, with an occurrence as high as 80% in Sprague Dawley rats, identified by elevated prolactin levels [Carretero et al., 2002; el Etreby et al., 1988; Kappeler et al., 2003; Umemura et al., 2001; van Putten et al., 1988].

MMQ cells, isolated from the prolactin-secreting rat pituitary tumor 7315a, are an interesting model since they only secrete prolactin. The Buffalo rat implanted with the prolactin-secreting MMQ tumor cells has been demonstrated to be an effective model for assessment of chronic hyperprolactinemia [Adler et al., 1991]. Also, the MMQ cell model has been used for evaluating the mechanisms involved in the modulation of prolactin release and could serve as a good model system to investigate gene therapy strategies aimed at inhibiting prolactin hypersecretion [Forget et al., 1993]. The purely prolactin secreting tumor 7315b has been shown to be a suitable model for studying the effects of severe hyperprolactinemia on the pituitary gonadal axis in rats, and can therefore be used as an *in vivo* model to test gene therapies aimed at reverting the affected phenotype [Kooy et al., 1989]. The 235-1 cell line derived from the transplantable rat pituitary tumor, 7315a, has been established in culture. When 235-1 was inoculated into athymic mice and rats of the Buffalo strain, tumors were produced, and the host animals had hypertrophied mammary glands [Reymond et al., 1984]. The SMtTW prolactin transplantable tumors in the Wistar/Furth rat have been shown to be an effective animal model that closely resembles the pathology found in the human prolactinomas [Trouillas et al., 1990]. Polysialylated neural cell adhesion molecule (PSA-NCAM), a highly expressed molecule during brain and pituitary development, exhibited high expression levels in the rat transplantable SMtTW pituitary tumors [Daniel et al., 2000]. Studies from this model raise the possibility of employing the PSA-NCAM molecule as a diagnostic marker for malignant pituitary adenomas. GH3 cells implanted into the flank of Wistar/Furth rats also constitute a good *in vivo* model of prolactinomas [McIntyre et al., 2004] and can be used to test both novel pharmacological therapies and gene therapy strategies. The SMtTW tumor, a spontaneous prolactin-secreting transplantable tumor is the only available animal model sensitive to dopamine agonists [Trouillas et al, 1994], it can therefore be used to test novel dopamine agonists and also gene therapies aimed at modulating endogenous dopamine levels [Williams et al., 2001].

GH Tumors

A model of GH hypersecretion was one of the first transgenic animal models generated [Palmiter *et al.*, 1982]. This mouse model has been used to analyze the effects of GH excess in various tissues and could be used to develop gene therapy strategies to normalize GH levels. One of the earliest molecular defects to be described in endocrine oncology involved single point mutations in two critical domains of the Gs protein, thereby maintaining Gs α in a constitutively activated state. These mutated G-proteins, also known as *gsps*, were first described in a subset of somatotroph adenomas and could be therapeupic targets using gene therapy techniques [Lyons *et al.*, 1990; Spada *et al.*, 1992].

The AVP-SV40T antigen model is another interesting model in which the expression of the SV40 T antigen, under the control of the arginine vasopressin (AVP) gene promoter, resulted in an animal model that developed a pituitary tumor [Stefaneanu *et al.*, 1992]. These anterior pituitary tumors are composed of undifferentiated somatotrophs. The intermediate lobe tumors were immunoreactive for ACTH and were positive for proopiomelanocortin (POMC) mRNA in a few cases.

Transplantable GH3 cells and GH3 cells in culture also constitute good models to evaluate novel therapies for GH secreting pituitary adenomas [Gruszka *et al.*, 2005; Kuchenbauer *et al.*, 2003; Lania *et al.*, 2004; Wasko *et al.*, 2004].

Cushing's Disease

Transgenic animals overexpressing corticotrophin releasing factor (CRF) exhibit endocrine abnormalities involving the hypothalamic-pituitary-adrenal axis, such as elevated plasma levels of ACTH and glucocorticoids. These animals display physical changes similar to those of patients with Cushing's syndrome. Studies with transgenic murine models have demonstrated to be very useful in the generation and characterization of Cushing's disease. Transgenic mice that developed ACTH-producing pituitary tumors were generated with the polyoma early region promoter coupled to a cDNA encoding polyoma large T antigen [Helseth *et al.*, 1992]. This study showed the development of microadenoma at 4 months and macroadenoma at 9 months. Indistinguishable morphology and ACTH immunoreactivity comparable to the parent tumor was observed in nontransgenic immunocompetent mice with subcutaneous transplants of pituitary tumor cells. The effects of hypercorticotropism were more enhanced and occurred with a shorter latency in the mice carrying pituitary transplants than in the polyoma large T antigen transgenic mice. The plasma ACTH levels were significantly increased in clinically ill transgenic mice and even higher levels were found in the transplanted mice.

To evaluate whether CRF overproduction leads to Cushing's syndrome and develop an animal model of chronic pituitary-adrenal activation, the CRF gene was expressed under the control of the metallothionine promoter in transgenic mice. Endocrine abnormalities involving the hypothalamic-pituitary-adrenal axis, such as elevated plasma levels of ACTH and glucocorticoids were observed in the CRF transgenic animals [Stenzel-Poore *et al.*, 1994].

Numerous cytokines, particularly leukemia inhibitory factor (LIF), are involved in the differentiation, proliferation, and secretory function of ACTH-producing pituitary cells. LIF and its receptor LIF-R are abundantly expressed in normal pituitaries and in ACTH producing adenomas. Transgenic mice expressing LIF under the regulation of the pituitary glycoprotein hormone α subunit promoter caused Cushing's syndrome [Yano *et al.*, 1998].

The neuroendocrine-specific protein 7B2 is required for the production of active prohormone convertase 2 (PC2), an important neuroendocrine precursor processing endoprotease. Mice null for 7B2 exhibit a lethal phenotype with a complex Cushing's-like pathology [Sarac *et*

al., 2002]. These mice constitute a good model system in which to attempt normalization of ACTH levels and elimination of the Cushing's phenotype.

Mice with a single copy of the retinoblastoma gene (Rb(+/−)) develop a syndrome of multiple neuroendocrine neoplasia. To prevent melanotroph neoplasia, transgenic mice in which a 770-bp fragment of pro-opiomelanocortin promoter directs expression of the human Rb gene to melanotrophs (TgPOMC-RB) was used. In three independent lines, transgenic mice crossed to Rb(+/−) background are devoid of melanotroph tumors but develop the usual spectrum of other neoplasms. Also, abrogation of melanotroph carcinogenesis results in accelerated progression of anterior pituitary lobe tumors [Zhou *et al.*, 2005]. These mice constitute a powerful *in vivo* model in which to test gene therapy approaches aimed at reverting the affected anterior pituitary lobe phenotype. Another potential model has been described, in which the Cre-LoxP mediated somatic inactivation of Rb in a subset of neuroendocrine cells, resulted in tumor of the pituitary and pineal gland [Vooijs *et al.*, 2002].

Also, AtT20 cells, which secret ACTH in culture, are a good model system to investigate treatment strategies aimed at inhibiting secretion and cell proliferation [Bahar *et al.*, 2004; Heany *et al.*, 2002; Lee *et al.*, 2001a; Simard *et al.*, 2002].

Null Cell Adenomas and Gonadotropinomas

Null cell adenomas do not synthesize anterior pituitary hormones but have shown to express gonadotrophin-related genes. Transgenic mice carrying a temperature-sensitive mutant (TSA58) of simian virus 40 T antigen driven by human follicle stimulating hormone-beta (FSHβ) regulatory elements were produced [Kumar *et al.*, 1998]. These animals developed slow growing, multifocal pituitary nodules that secrete FSH. Anterior pituitary pathology progressed from diffuse gonadotroph hyperplasia to nodular adenomas with persistent, but decreasing immuno reactivity for FSHβ and luteinizing hormone β-subunit (LHβ). Ultrastructural characteristics of the tumors were identical to those of human null cell adenomas. Therefore, this is a good animal model for further study of this disease. Also LbetaT2 and alpha T3 gonadotrophs-derived cell lines are useful models to study physiopathology of the gonadotroph function [Laverriere *et al.*, 2004; Nilson *et al.*, 2000].

Although most pituitary tumor cell lines *in vitro* have a high rate of doubling time, and the same characteristic is seen when these cells are transplanted in the periphery to generate tumors, they constitute an adequate model to test therapies that target pituitary tumor growth and invasiveness. They are also adequate to test the safety, toxicity and biodistribution of gene therapy vectors. Also, one can test adverse immune responses to the vectors and long term therapeutic transgene expression for future clinical implementation. Of course one has to take the limitations described above into account before embarking in clinical trials and also in predicting clinical outcome.

Transgenic or knockout technologies, as well as the other animal models mentioned, are important tools to examine the regulation of hormone gene expression and the pathophysiology of its alterations. Additional studies on these and other tumor models are necessary to determine an optimal animal model that allows us to examine novel therapeutic strategies for the management of patients with pituitary disorders.

PRECLINICAL STUDIES

Preclinical trials using several gene therapy approaches for the treatment of anterior pituitary diseases have been developed during the last decade. Successful transgene delivery to the anterior pituitary gland has been achieved using first generation recombinant adenoviral vectors (RAds) [Castro *et al.*, 1997; Lee *et al.*, 2001a; Lee *et al.*, 2001b; Southgate *et al.*,

2001; Williams *et al.*, 2001; Windeatt *et al.*, 2000;], herpes simplex virus type 1 (HSV-1) derived vectors [Carri *et al.*, 2005; Goya *et al.*, 1998] and lentiviral vectors [Roche *et al.*, 2004].

RAd-encoded transgenes have been successfully expressed within all the cell types of the adenohypophysis and in the pituitary tumoral cells AtT20 and GH3 [Castro *et al.*, 1997]. Although long term expression can be achieved in normal and tumoral pituitary cells, the persistence of transgene expression seems to be cell cycle-dependent, being longer in slowly dividing cells than in actively dividing cells. On the other hand, the efficiency of infection is higher in actively dividing GH3 cells compared with growth-arrested GH3 cells. Gene transfer to the pituitary gland was also achieved *in vivo* by intrapituitary administration of RAds. Transgene expression within the anterior pituitary gland after the local administration of RAds have been detected for up to 3 months post viral delivery in rats [Southgate *et al.*, 2001]. Although transgene expression declined over time, the persistence of adenoviral genomes is observed for up to 3 months. A cellular immune response against the virus was described, characterized by local infiltration of macrophages, CD8+ T-cells, and NK cells for up to 1 month after virus administration [Southgate *et al.*, 2001]. Systemic anti-adenovirus neutralizing antibodies were also detected 14 days after virus administration.

The use of the latest developed high capacity adenovirus, which is devoid of all viral coding sequences, will improve adenovirus-mediated gene therapy in the pituitary gland, as was shown in other tissues [Hardy *et al.*, 1997; Kochanek *et al.*, 1996; Lowenstein *et al.*, 2002; Mitani *et al.*, 1995; Parks *et al.*, 1996; Parks *et al.*, 1999; Umana *et al.*, 2001]. High capacity adenoviral vectors have a large cloning capacity and elicit very long term and stable transgene expression with very low toxicity in the central nervous system and peripheral organs. These vectors are able to transfer and direct expression of therapeutic genes *in vivo* with unsurpassed efficiency among gene therapy vectors [Lowenstein *et al.*, 2002]. Further, since the genome of these vectors remains episomal there are no risks of insertional mutagenesis or oncogene activation as has been reported with other gene transfer vectors [Baum *et al.*, 2004; Kustikova *et al.*, 2003].

Although RAds have been the most extensively used vectors for pituitary gene delivery, HSV-derived vectors [Carri *et al.*, 2005] and lentiviral vectors [Roche *et al.*, 2004] have also shown to be efficient tools for transgene delivery to the pituitary. HSV-derived vectors encoding β-galactosidase as the reporter gene succesfully transduced pituitary cells *in vivo* after the local administration of the vector [Carri *et al.*, 2005]. Human pituitary gonadotroph and somatotroph adenoma cells were transduced *in vitro* using a human immunodeficiency virus (HIV)-type 1-derived vector encoding the enhanced green fluorescent protein gene under the phosphoglycerate kinase promoter [Roche *et al.*, 2004].

Transcriptional Targeting

The feasibility of targeting specific pituitary cell types *in vivo* makes the cell specific therapeutic approach a useful tool to treat different pituitary disorders. Recombinant adenoviruses containing the GH promoter [Lee *et al.*, 2000], the glycoprotein hormone α-subunit promoter [Lee *et al.*, 2000], the POMC promoter [Lee *et al.*, 2001a] and the prolactin promoter [Castro *et al.*, 1997; Davis *et al.*, 2001b; Southgate *et al.*, 2001] were used to selectively drive transgene expression in specific endocrine pituitary cell populations in animal models. Although the systemic administration of RAds containing the GH or α subunit promoter failed to deliver the marker gene to the pituitary in rat models, direct stereotactic injection of recombinant adenoviral vectors into the pituitary achieved a high level of selective transgene expression in pituitary cells that normally produce the respective hormones [Lee *et al.*, 2000; Lee *et al.*, 2001a; Southgate *et al.*, 2001]. The expression of lentivirus was also targeted to pituitary cell subpopulations by pituitary hormone promoters, showing that the

glycoprotein hormone α subunit promoter was selectively transduced in gonadotropes and tyrotropes [Roche *et al.*, 2004]. Although transgene expression is lower under the control of the GH and prolactin promoters compared to the ubiquitous CMV promoter, anterior pituitary hormone promoters allow endocrine pituitary cell specificity [Roche *et al.*, 2004; Southgate *et al.*, 2001].

The incorporation of inducible promoter systems into viral vectors allows switching transgene expression "on" and "off", which becomes specially critical if adverse side effects develop due to the therapy. The incorporation of regulatory systems into viral vectors has allowed for tight regulation of expression of the encoded transgenes in the pituitary gland [Smith-Arica *et al.*, 2001; Williams *et al.*, 2001]. Lactotrophspecific inducible transgene expression was achieved in the rat pituitary gland following the intrapituitary administration of a dual adenoviral vector system, in which the tetracycline-responsive transcriptional elements have been engineered to be under the control of the human prolactin promoter [Smith-Arica *et al.*, 2001]. Transgene expression was shown to be turned off and on by the administration or withdrawal of doxycycline, respectively, suggesting that combined transcriptional and inducible transgene expression can be achieved using adenoviral vectors that allow spatial and temporal restriction of transgene expression within the anterior pituitary gland.

Pituitary Gene Therapy: Safety and Toxicity

The safety of the intrapituitary administration of adenoviral vectors remains controversial. Davis *et al.*, reported that in sheep the intrapituitary administration of a dose of 15×10^8 pfu of viral vector encoding β-galactosidase in a volume of 2.5 ml displayed varying degrees of inflammation, including periglandular fibrosis, lymphocytic infiltrate, venulitis and necrosis/apoptosis foci [Davis *et al.*, 2002]. Although inflammatory cells were reported to infiltrate the transduced area [Smith-Arica *et al.*, 2001], a severe inflammatory response was not observed by studies of gene transfer in rodents and may rely in the dose and volume used in Davis' report. In fact, a recent report shows that the intrapituitary injection of 5×10^5 pfu of RAd or HSV-1-derived vectors in a volume of 1 µl results in successful expression of the transgene without any sign of toxicity in the rat [Carri *et al.*, 2005]. The expression of β-galactosidase driven by adenoviral as well as HSV-1-derived vectors injected directly in the rat pituitary was reported to preserve cellularity, lactotrophic cell density and apoptotic levels. These observations were similar to those seen in the saline injected rats and did not affect prolactin or GH serum levels [Carri *et al.*, 2005]. More so, the intrapituitary administration of 1×10^8 infectious units of adenoviral vectors in rats showed no deleterious effects on circulating levels of anterior pituitary hormones up to three months post viral delivery [Southgate *et al.*, 2001]. Thus, although the intrapituitary administration of viral vectors seems to be safe, close attention must be paid to the vector dose before applying these therapies in humans.

Therapeutic Genes

Although the low diffusibility of viral vector throughout the pituitary tissue has been proposed to constitute a drawback for achieving complete remission of pituitary tumors [Carri *et al.*, 2005; Davis *et al.*, 2001b; Southgate *et al.*, 2001], several transgenes have been shown as promising gene therapy treatments for pituitary adenomas. HSV-1 thymidine kinase (HSV1-TK) converts the prodrug ganciclovir (GCV) to a nucleotide that is highly toxic to proliferating cells. Adenoviral vectors encoding HSV-TK have been developed and tested in preclinical trials for the treatment of pituitary tumors [Lee *et al.*, 1999; Windeatt *et al.*, 2000] and Cushing's disease [Lee *et al.*, 2001a]. The intrapituitary administration of adenoviral vectors encoding HSV-TK to estrogen-induced lactotroph hyperplasia-bearing rats followed by the systemic administration of GCV resulted in decreased prolactin plasma levels and reduction in the size of the pituitary gland [Windeatt *et al.*, 2000]. GH3 tumors developed in the flank of nude mice showed remarkable reduction after the intratumoral administration of a RAd encoding HSV-

TK under the control of GH promoter in only 10 days [Lee *et al.*, 1999]. In a mouse model of Cushing's disease, induced by AtT20 cell implantation into the flank of nude mice, the intratumoral injection of an adenoviral vector encoding HSV-TK under the control of POMC promoter caused significant tumor regression [Lee *et al.*, 2001b].

Hypotalamic dopamine has been extensively reported to inhibit lactotroph growth [Duvilanski *et al.*, 1996] as well as prolactin secretion [George *et al.*, 1979; Kalbermann *et al.*, 1979; Kalbermann *et al.*, 1980; Lloyd *et al.*, 1975; Perez *et al.*, 1986]. The expression of tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, in the rat anterior pituitary gland has been successfully induced by intrapituitary injection of a dual adenovirus tetracyclineregulatable expression system [Williams *et al.*, 2001]. The *in vivo* expression of TH was produced in lactotrophs, somatotrophs, corticotrophs, thyrotrophs, and gonadotrophs and was shut off by the presence of doxycycline. In a model of estrogen-induced pituitary tumor, the adenoviral vector encoding TH exerted, in the absence of doxycycline, a 50% reduction in pituitary growth and 60% reduction in the increased circulating levels of prolactin. These effects were not observed in rats treated with estrogen and sulpiride, a dopaminergic antagonist, showing the specificity of this gene therapy approach [Williams *et al.*, 2001].

Diphtheria toxin has also been used as a target for suicide gene therapy for pituitary tumors. The diphtheria toxin gene was successfully targeted to GH4 tumors transplanted to nude mice using adenoviral vectors which caused rapid regression of the tumor [Lee *et al.*, 2002], suggesting that this approach could be a useful tool for the treatment of GH-secreting adenomas as well as other neoplastic disorders.

Considering that estradiol plays a key role in lactotroph function, proliferation, differentiation, and death, the estrogen receptor (ER) function was manipulated in pituitary cell lines using gene therapy approaches. The infection of GH4 cells with adenoviral vectors encoding a dominant negative ER mutant that impairs the activation of endogenous ER, results in the inability of these cells to form tumors in nude mice [Lee *et al.*, 2001b], suggesting that dominant negative ER mutations could serve as a therapy for pituitary lactotrope adenomas as well as other estrogen-responsive tumors.

Recent work from our group reveals that the overexpression of proapoptotic factors could be a useful tool in the therapy of pituitary adenomas. RAds encoding TNF-α or FasL successfully transduced anterior pituitary cells as well as GH3 and AtT20 cells, increasing the percentage of hypodiploid and TUNEL positive cells [Candolfi *et al.*, 2005]. In primary cell cultures the proapoptotic effect of TNF-α and FasL gene transfer was observed in lactotrope and somatotrope subpopulations. *In vivo* studies, such as intrapituitary gene transfer to pituitary adenoma-bearing rats are necessary to further determine the therapeutic potential of this approach.

RAds have been used in preclinical trials not only to treat pituitary adenoma, but also to treat pituitary endocrinopathies. In a mouse model of Cushing's disease, the replacement of the chaperone protein 7B2 was achieved by a single intrapituitary injection of 10^6 pfu, resulting in a drop in circulating ACTH levels, an increase in serum glucose levels, a reduction of the high systemic levels of corticosterone and prolonged survival times [Sarac *et al.*, 2002]. This study shows the feasibility of replacing deleted or abnormal genes by gene therapy approaches, which could prove to be a useful tool for the treatment of anterior pituitary insufficiencies.

CONCLUSION

Although gene therapy requires further investigation of its efficiency and safety to be used for the treatment of pituitary diseases in humans, the versatility of this technique offers strong rational for the development of new therapeutic tools for a wide range of applications. These

include tumor regression, supplementation of abnormal genes and normalization of hormone secretion. When pituitary tumors do not respond to current therapeutic strategies, pharmacological therapy is not well tolerated, or the tumor becomes aggressive or difficult to treat over time, gene therapy could be an attractive treatment modality. Carefully designed and controlled clinical trials may assess the potential of gene therapy as a useful treatment option for currently difficult-to-treat pituitary tumors such as Cushing's or non-secreting macroadenomas. Gene therapy offers molecular interventions which should be capable to achieve complete elimination of tumor cells after surgical tumor removal, without affecting surrounding normal pituitary tissue and also normalization of hormone hypersecretion. One would envisage that gene therapy will be used in combination with the best current treatment options available and it will provide the possibility of targeting receptors, signal transduction pathways and tumorigenic mechanisms which currently can not be targeted by classical pharmacology and/or radiotherapy. Thus, combining current treatments with gene therapy could provide long-term cure for most pituitary tumors. The field would need to advance further before gene therapy becomes a clinical reality for the treatment of pituitary tumors, especially in relation to its safety and putative immune responses, since these adverse side effects could have a very deleterious impact on the function of the healthy pituitary cells remaining. In spite of these hurdles, it constitutes one of the most promising approaches to develop future novel therapies for pituitary adenomas and tumors located in the sella turcica.

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Table 1

Classification of Pituitary Tumors

Subtype	% of pituitary tumors	Hormone expression	Principal clinical manifestations
Prolactinomas	25–41	PRL	Galactorrhea, hypogonadism.
Somatotroph adenomas	10–20	GH (GH + PRL)	Acromegalia
Corticotroph adenomas	5–15	ACTH	Cushing's syndrome (hypercortisolism)
Gonadotroph adenomas	10–15	FSH, LH, alpha subunit	menstrual irregularities, hypopituitarism, mechanical effects
Thyrotroph adenomas	0.5–2	TSH, alpha subunit	hyperthyroidism
Null-cell adenomas	5–10	None	mechanical effects, hypopituitarism

Table 2

Gene Transfer Vehicles Used in Gene Therapy Applications

	RAd	HC-Ad	HSV-1/r	HSV-1/a	AAV	Retrovirus
Particle size (nm)	80–120	80–120	120–300	120–300	20–30	100
Genome size (kb) Genome type	36 (dsDNA)	30–36 (dsDNA)	152 (dsDNA)	152 (dsDNA)	4.68 (ssDNA)	3.5–9.2 (ssRNA)
Cloning capacity (kb)	8.0	~30	30–50	150	2–4.5	~8
Transduction *In vivo* *In vitro*	yes yes	yes yes	yes yes	yes yes	yes yes	yes yes
Long term expression	no	yes	yes	yes	yes	yes
Vaccination	yes	yes	yes	yes	yes	yes
Stock vector titers (transducing units/ml)	10^{12}	10^{11}	10^8	10^8	10^9	10^7

RAd, recombinant adenovirus; HD-Ad, helper-dependent adenovirus vector; HSV-1/r, herpes simplex type 1 recombinant vector; HSV-1/a, herpes simplex type 1 amplicon; AAV, recombinant adeno-associated virus; transducing units/ml, number of transgene expressing particles per milliliter; dsDNA, double strain DNA; ssDNA, single strain DNA; ssRNA, single strain RNA.

Table 3

Advantages and Disadvantages of Viral Vectors for Gene Therapy

Vector	Advantages	Disadvantages
Enveloped		
HSV/r	Broad cell tropism, high tropism for neurons latency in neurons, very stable	Expression of viral genes
		Highly toxic
		Transient transgene expression in cells other than neurons
HSV/a	Broad cell tropism, high tropism for neurons latency in neurons, very stable	No expression of viral genes
	Large transgene capacity	Lower toxicity
		Infects dividing cells only
Retrovirus	Persistent transgene expression	Integration into host genome
		Risk of insertion mutagenesis
Lentivirus	Broad cell tropism	Integration into host genome
	Infection of dividing and non dividing cells	Risk of insertion mutagenesis
	Persistent transgene expression	Risk of seroconversion
Non-enveloped		
Rad	Broad cell tropism, infection of dividing and non dividing cells	Expression of viral proteins
	High titer stocks, good yields	Inflammatory and immune responses
		Transient expression
		RCA potential contamination
HD-Ad	Broad cell tropism, infection of dividing and non dividing cells	Difficulty in large scale production
	Non expression of viral proteins	Helper virus and RCA potential contamination
	Lower inflammatory and cellular immune response	
	Longer term transgene expression	
AAV	Broad cell tropism	Small insertion capacity
	Infection of dividing and non dividing cells	Integration into host genome
	Non expression of viral proteins	
	Discrete immune response	