Cell-type specific expression in the pituitary: physiology and gene therapy

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Introduction: pituitary tumours
Pituitary tumours are very common, being present in approximately 11% of population at autopsy. Thus most are of little clinical significance and only a minority lead to clinical symptoms. They are usually classified clinically by the hormone(s) or hormone subunits which they secrete, and their size, i.e. micro (< 10 mm in diameter) or macro-adenomas (> 10 mm in diameter) [1]. Small pituitary tumours may cause severe clinical symptoms due to hormone hypersecretion and large tumours can give rise to problems related to the displacement of normal surrounding brain structures, i.e. compression of the optic chiasm, invasion of the hypothalamus and/or the pituitary stalk. Some tumours might also invade further adjacent brain structures.

Current treatments
The main goals of the treatment of pituitary tumours include: (i) reduction or elimination of the tumour mass; (ii) normalization of the hypersecretion of hormones produced by the tumour; (iii) the maintenance of remaining pituitary functions; (iv) limitation and prevention of damage to neighbouring structures; and (v) the correction of hormonal deficits due to tumour damage to surrounding normal pituitary tissue. Trans-sphenoidal surgery is the treatment of choice for most pituitary tumours [2], with the exception of prolactinomas. Radiation therapy has varying efficacy in reducing tumour size and hormone levels, and it also has substantial adverse side-effects, i.e. hypopituitarism, and in some patients the occurrence of central-nervous-system tumours has been reported [3]. This form of therapy is reserved for patients not cured after surgery and/or pharmacological treatment. Receptor-mediated pharmacotherapy has also been developed and is the treatment of choice for prolactinomas. Dopaminergic agonists such as bromocriptine have been successfully used for normalizing prolactin levels and also reducing tumour mass [4]. Dopaminergic drugs can also reduce growth hormone (GH) levels in patients with acromegaly. The somatostatin analogue, octreotide, can also reduce GH levels in patients with GH-producing pituitary tumours [5,6]. Octreotide is also useful for the treatment of patients with thyroid-stimulating-hormone-secreting tumours [7]. In patients with adenocorticotrophic hormone-secreting pituitary tumours, pharmacological therapies have been much less successful at reducing hormone levels and tumour size [8].

In spite of the dramatic advances in pituitary tumour treatment using classical therapeutic modalities, there are a number of patients that either do not respond or do not tolerate pharmacological treatment [9]. Also some tumours recur after pharmacological or surgical treatment [10]. Therefore, for some pituitary tumours that are large, locally invasive, endocrine-active and non-responsive to the pharmacological treatments, cure using currently available treatments has proven impossible to achieve.

Below we will highlight possible gene-therapy strategies that might be useful for the treatment of such tumours, which take advantage of pituitary cell-type-specific expression of therapeutic genes (Table 1).

Development of novel treatment strategies based on gene therapy
Gene therapy can be defined as the use of nucleic acids with therapeutic intent. It can be considered as part of pharmacology since the same principles and side effects that apply to classical pharmacological treatments can be applied to gene-based therapies. Although gene therapy is still at an early stage of development, and there have been no major breakthroughs in terms of successful therapeutic outcomes in humans, there are very high expectations from this new therapeutic modality based on results from in vitro, in vivo models of disease, and also human trials (for reviews, see [11–15]). The transfer of therapeutic genes can be achieved using physical or virally mediated approaches. In most of the clinical gene-therapy protocols approved to date, the therapeutic gene is targeted to somatic cells and, therefore, the genetic
information cannot be transferred to the progeny of the subject.

As far as methods for therapeutic gene delivery are concerned, there are two modalities: transplantation of autologous cells which have been genetically engineered \textit{ex vivo}, or direct \textit{in vivo} delivery of the therapeutic gene into the target tissue/organ. Currently, \textit{in vivo} approaches are gaining popularity with respect to \textit{ex vivo} methods [11].

\textbf{Viral vectors for gene delivery}

There are many different viral vectors that have been used for \textit{in vitro} and \textit{in vivo} delivery. The viral vector of choice will depend on the therapeutic target, i.e. for Parkinson’s disease long-term transgene expression will be necessary. Thus, a vector capable of integrating into target-tissue chromosomes would be advantageous, whereas for cancer treatment transient expression would be adequate so an episomal vector could be used. The most commonly used viral vectors include retrovirus [16], lentivirus [17], adeno-associated virus [18], adenovirus [19] and herpes virus [20]. In our laboratory we are currently using adenoviruses for gene delivery into the pituitary gland [21] and this review will concentrate on these vectors.

The method originally used for the production of first-generation adenoviruses involves the use of two plasmids; one shuttle vector containing the expression cassette flanked by adenoviral sequences and a plasmid containing the rest of the adenoviral genome with deletions in the E1 and E3 regions. The plasmid containing the adenoviral genome cannot be packaged into viral particles because it either contains an insert of pBR322, which makes the plasmid too large, or it has the packaging sequence removed.

The deletion in the E1 region renders the adenoviral vector replication-incompetent because the viral transactivators, which activate the expression of the other viral early genes, are removed. The two plasmids are co-transfected using the calcium phosphate transfection method into 293 cells [22]. These are derived from human embryonic kidney cells and express the deleted E1 region \textit{in trans}, so the adenovirus can replicate within these cells. Adenoviral vectors are generated when the two plasmids undergo homologous recombination within the transfected 293 cells (Figure 1). The deletion in the E1 region allows the insertion of approximately 5 kb but combined with the deletion in the E3 region 8 kb of foreign DNA can be inserted.

Another method used for generating adenoviral vectors with inserts of up to 10 kb [23] is to perform the plasmid recombination in a suitable strain of \textit{Escherichia coli}. This method is advantageous because it allows the screening of the bacterial clones for the viral DNA required, using resistance to the antibiotic kanamycin as a marker, before generating the virus in 293 cells. One can therefore be sure to have the correct viral DNA construct, which contains the expression cassette, and that only one viral clone will be generated.

A new method for producing adenoviral vectors containing no viral genes, ‘gutless’ adenovirus, has also been described [24]. These vectors consist of the inverted terminal repeats, the packaging sequences and stuffer DNA. They have an insert capacity close to 28 kb. To generate gutless recombinant adenovirus an E1-deleted helper virus is needed to complement the missing viral gene functions. \textit{lox-P} sites flank the packaging sequence within the helper virus. As the 293 cells in which these viruses are grown express Cre recombinase, which excises the packaging signal flanked by \textit{lox-P} sites, the helper virus can be
removed from the preparation. These new gutless adenoviral vectors show promise for enhanced safety, prolonged transgene expression and reduced immunogenicity (reviewed in [25]).

**Targeted gene expression within the anterior pituitary gland using recombinant adenoviral vectors**

Modification of viral tropism

We have previously shown that herpes simplex virus type I and adenoviruses are efficient for transferring genes into the anterior pituitary gland in vitro [21,26]. For efficient and safe gene transfer in vivo it would be useful to generate adenoviral vectors that could target and infect certain cell populations. The adenovirus has a broad tropism allowing it to infect a wide variety of cells. The adenovirus interacts with two cellular receptors; the fibre protein of the virus capsid binds to the CAR (coxsackie and adenovirus receptor) in a primary interaction, which is followed by the binding of an RGD sequence motif in the penton base to \( \alpha_\text{v} \) integrins. One way of modifying the knob is to use an anti-knob monoclonal antibody that is chemically conjugated to a ligand recognizing a specific cellular receptor. This has been demonstrated using the folate receptor [27], which is overexpressed in several carcinomas including breast and lung cancers. Fibroblast growth factor (FGF2) has also been used to target adenoviral vectors to the FGF receptor (FGFR) [28]. Recently Gu et al. have shown that the adenovirus retargeted to the FGFR have a 10–100-fold increase in gene expression in FGFR-positive cells when compared with adenovirus [29]. In vivo data show decreased hepatic toxicity and liver transgene expression. Also they showed enhanced survival in mice containing adenovirus-resistant FGFR-positive tumour cells when treated using an FGF2-retargeted adenovirus coding for herpes simplex virus type-I thymidine kinase in conjunction with ganciclovir treatment. One draw-

**Figure 1**

Generation of a recombinant adenoviral vector

(A) The shuttle vector containing the expression cassette and the plasmid containing the adenoviral (Ad) genome (pBHGI10; black) are cotransfected into 293 cells. After homologous recombination between the two plasmids a replication-incompetent adenovirus is produced. The expression cassette (B) can be produced using different promoters, e.g. the prolactin (Prl) promoter, to control the expression of a marker gene, a cytotoxic gene or another therapeutic gene of interest.
back to using this approach is that the antibody could dissociate from the viral particle as it is not covalently linked.

Another approach involves genetically modifying the knob domain of the fibre protein by the incorporation of a ligand specific for a cellular receptor. A restriction of this approach is that there are structural limitations for the fibre conformation to be taken into consideration. Genetic modifications to the C-terminus of the knob domain have been shown to alter the adenoviral tropism. Wickham et al. have constructed two adenoviral vectors with sequences coded into the fibre knob [30]; (i) with a heparin-binding domain, which is expressed in many cell types, and (ii) with an RGD sequence, which redirects the adenoviral binding to the $\alpha_i$ integrins. Another similar approach involves incorporation of a heterologous peptide within the structure of the knob [31]. These approaches suggest that fibre modification is a viable strategy to modify viral tropism but viral receptor binding will have to be inhibited by knob mutagenesis to achieve true targeting.

Wickham et al. have replaced the RGD motif in the penton base, which mediates the internalization of the adenovirus by binding to specific integrins, with a FLAG peptide epitope. Using a bispecific antibody recognizing the FLAG epitope and either the $\alpha_i$ integrin [32] or human CD3 [33], the targeting of the adenovirus can be altered to infect cells that are normally poorly transduced by adenoviral vectors.

The change in viral tropism could be useful when treating pituitary tumours so the viral infection can be targeted to certain cell populations, i.e. lactotrophs, somatotrophs or corticotrophs. The receptor to be targeted should be tumour-dependent, as every tumour will overexpress different receptors. One possible target is the GH secretagogue receptor, a member of a family of transmembrane receptors. It has been shown that the expression of the GH secretagogue receptor is increased 200-fold in GH adenomas and 10-fold in thyroid-stimulating-hormone-producing adenomas [34]; this receptor could be a target for these tumours.

Other potential targets are the vasopressin (V3) and corticotrophin-releasing hormone receptors that are overexpressed in corticotroph tumours [35]. The more invasive tumours have been shown to overexpress epidermal growth factor receptor [36], another potential target receptor. Finally it has been demonstrated that some pituitary adenomas have altered FGFR subtypes compared with the normal pituitary [37]. This leads to the possibility of using one of these subtypes, not normally expressed in the pituitary, to target the adenomatous tissue.

Transcriptional targeting using cell-type-specific promoters

Another possibility for targeting specific cell types in the pituitary is to use cell-type-specific promoters. Although all cell populations will be infected the expression of the transgene will be restricted to specific cells. The need for cell-type-specific expression is important for expression of toxic transgenes, as is the case for cancer-gene therapy. The prolactin promoter has been demonstrated to restrict transgene expression to the lactotroph and mammosomatotroph cells [38]. In our laboratory we have shown that an adenoviral vector can express transgenes in a cell-type-specific manner under the control of the human prolactin promoter [39]. For the treatment of somatotroph tumours, Lee et al. [40] have shown that cell-type-specific expression can be obtained using the GH promoter controlling transgene expression. Other promoters that could be used include the pro-opiomelanocortin promoter for corticotroph tumours [41], the thyrotropin $\beta$ promoter for thyrotrope-specific expression [42] and the gonadotropin-releasing-hormone receptor promoter to restrict expression to gonadotropes [43].

Development of cell-type-specific and regulatable recombinant adenoviral vectors for transgene expression within the anterior pituitary gland: applications for pituitary tumour treatment

Within the field of gene therapy it is becoming increasingly clear that regulation of transgene expression from delivery vehicles such as adenoviruses would be advantageous in certain therapeutic paradigms. There are now numerous regulatory systems available, which have been shown to be functional from viral vectors; however, the one that has received the most attention is the tetracycline-inducible system (tet system) [44].

The tet system is based upon the tetracycline-resistance transposon Tn10. In this system the tetracycline transactivator (tTA), in the absence of the inducer doxycycline binds to a tetracycline response element and drives the expression of the desired transgene. However, when the inducer is added the tTA dissociates from the transactivator, resulting in the cessation of further transgene expression; this is termed the tet-off system.
Conversely, by the introduction of four point mutations in the transactivator, a tet-on system \cite{45} has been developed where the inducer activates transgene expression.

The tet system has been shown to be functional from adenoviral vectors both \textit{in vitro} \cite{46} and \textit{in vivo} \cite{47}, allowing the tight regulation of transgene expression. We and others have modified the tet system, restricting the inducible expression to certain sub-populations of cells. Initial studies, using the glial fibrillary acidic protein (GFAP) promoter \cite{48} to drive the expression of the tTA in adenovirus vectors, showed that tetracycline-inducible expression could be restricted to permissive cell lines of glial lineage. In our laboratory, we have shown that by driving the expression of the tTA by the prolactin-specific promoter from adenoviral vectors, we can restrict inducible transgene expression to both lactotrophic tumour cell lines and prolactin-positive cells in primary anterior pituitary cultures \cite{49}.

The development of a prolactin-specific and inducible expression system in adenoviral vectors potentially has applications for the treatment of human prolactinomas. By expressing cytotoxic transgenes or immunomodulatory proteins from our system, expression cannot only be restricted to the target cell type but can also be regulated by the administration of the inducer (Figure 2). Such a system would provide safer and more efficient transgene delivery, putting therapeutic control in the hands of the clinician, thus circumventing any possible toxic side effects of the therapy.

**Conclusions**

The field of gene therapy is progressing rapidly and is gaining consensus for its application as an alternative treatment strategy for diseases that do not respond to current treatments, such as cancer and neurodegenerative disorders. Although pituitary tumours are usually benign, some are aggressive and do not respond to classical treatment strategies. We therefore propose that gene therapy would be a suitable alternative therapeutic option. Ongoing clinical trials and toxicity studies should provide us with important information to progress these approaches for the treatment of human pituitary tumours.

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**References**

Gene Therapy: from Bench to Bedside


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