



Published in final edited form as:

*Curr Gene Ther.* 2004 March ; 4(1): 79–87.

## Potential of Gene Therapy for the Treatment of Pituitary Tumors

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### Abstract

Pituitary adenomas constitute the most frequent neuroendocrine pathology, comprising up to 15% of primary intracranial tumors. Current therapies for pituitary tumors include surgery and radiotherapy, as well as pharmacological approaches for some types. Although all of these approaches have shown a significant degree of success, they are not devoid of unwanted side effects, and in most cases do not offer a permanent cure. Gene therapy—the transfer of genetic material for therapeutic purposes—has undergone an explosive development in the last few years. Within this context, the development of gene therapy approaches for the treatment of pituitary tumors emerges as a promising area of research. We begin by presenting a brief account of the genesis of prolactinomas, with particular emphasis on how estradiol induces prolactinomas in animals. In so doing, we discuss the role of each of the recently discovered growth inhibitory and growth stimulatory substances and their interactions in estrogen action. We also evaluate the cell-cell communication that may govern these growth factor interactions and subsequently promote the growth and survival of prolactinomas. Current research efforts to implement gene therapy in pituitary tumors include the treatment of experimental prolactinomas or somatomammotropic tumors with adenoviral vector-mediated transfer of the suicide gene for the herpes simplex type 1 (HSV1) thymidine kinase, which converts the prodrug ganciclovir into a toxic metabolite. In some cases, the suicide transgene has been placed under the control of pituitary cell-type specific promoters, like the human prolactin or human growth hormone promoters. Also, regulatable adenoviral vector systems are being assessed in gene therapy approaches for experimental pituitary tumors. In a different type of approach, an adenoviral vector, encoding the human retinoblastoma suppressor oncogene, has been successfully used to rescue the phenotype of spontaneous pituitary tumors of the pars intermedia in mice.

We close the article by discussing the future of molecular therapies. We point out that although, gene therapy represents a key step in the development of molecular medicine, it has inherent limitations. As a consequence, it is our view that at some point, genetic therapies will have to move from exogenous gene transfer (i.e. gene therapy) to endogenous gene repair. This approach will call for radically new technologies, such as nanotechnology, whose present state of development is outlined.

### Keywords

Gene therapy; viral vectors; pituitary tumors; estrogen; prolactinomas; neurosurgery; suicide gene therapy; combined therapy; nanotechnology

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## INTRODUCTION

Pituitary adenomas constitute the most frequent neuroendocrine pathology in humans, comprising up to 15% of primary intracranial tumors [Daniels and Martin, 1995]. Also, pituitary tumors represent the most prevalent pathology in old female rats [Burek, 1978]. Clinical manifestations of pituitary adenomas arise from overproduction of hormones in microadenomas or from mass effects in larger tumors.

There are five types of hormone-producing pituitary adenomas, which reflect the cell type that originated the tumor. These tumor types are prolactin (PRL)- and growth hormone (GH)-secreting tumors that cause reproductive abnormalities and the syndromes of acromegaly, respectively; corticotropin (ACTH)-producing tumors that cause Cushing's disease; and gonadotropin- and thyrotropin-secreting tumors that cause abnormalities in their respective axes. Mass effects of enlarging tumors usually include visual field defects resulting from compression of the optic nerves, headaches, hypopituitarism and, rarely, invasion into the skull base causing multiple intracranial nerve palsies. Only exceptionally are pituitary tumors truly malignant with distant metastases.

Current therapies for pituitary tumors include surgery and radiotherapy, as well as pharmacological approaches for some types [Shimon and Melmed, 1998]. Transsphenoidal surgery has proven to be highly effective for microadenomas but not for macroadenomas [Melmed, 1997]. In both cases post-surgical complications, like the appearance of a new pituitary dysfunction, diabetes insipidus and sometimes fistula formation, develop even in patients operated on by specialist surgeons. Pharmacological therapies, with dopamine agonists like bromocriptine, cabergoline and quinagolide, have met with remarkable success in shrinking prolactinomas and reducing the hyperprolactinemia associated with them [Bevan *et al.*, 1992]. Unfortunately, a significant rate of side effects like nausea, vomiting, postural hypotension, dizziness, headaches, and constipation is present in long-term treatments with these drugs [Webster *et al.*, 1994]. In patients with GH-producing adenomas, somatostatin analogs and depot preparations of octreotide can lower the usual hypersomatotropinemia to levels compatible with acceptable long-term survival [Sheppard, 1994]. Treatment with octreotide of 27 newly diagnosed acromegalic patients for 6 months, followed by treatment with a long-acting octreotide preparation for additional 6 months, resulted in 79% of the patients having mean serum GH levels below 5 mU/liter, 53% having normal IGF-I levels, and 73% of the patients showing greater than 30% tumor shrinkage (Bevan, 2002). None of the aforementioned pharmacological treatments cure the tumors, which will usually recur if the medications are discontinued.

Radiotherapy is usually used as an adjunctive treatment after pituitary tumor surgery. Although long-term irradiation is often effective to prevent tumor regrowth, it usually causes complications, the most frequent (58 to 83%) of which is hypopituitarism (Sutton, 1985).

Other less frequent complications associated with conventional radiotherapy for pituitary adenomas are 2% optic chiasmal injury and 0.2% radiation brain necrosis (Sutton, 1985). Although rare, radiation-induced fibrosarcomas and osteosarcomas have been reported (Grigsby and Sheline, 1990).

In summary, although important advances have been made in the treatment of pituitary tumors, a fully satisfactory therapy is not yet available. In this context, gene therapy appears potentially useful as an alternative for the treatment of pituitary tumors. Although research efforts to apply this methodology to the hypophysis are relatively recent, promising experimental results, discussed later in this review, have already emerged.

## GENESIS OF PITUITARY TUMORS

The genesis of pituitary tumors is multifactorial. Since PRL-secreting adenomas (prolactinomas), the more prevalent type of pituitary tumors, are the most studied regarding their etiology, we will focus our discussion on the genesis of this particular type of pituitary tumor.

Prolactinoma development involves clonal expansion. Some of the contributing genetic events include H-Ras mutation for invasive prolactinomas [Karga *et al.*, 1992] and partial loss of chromosome 11, including the putative multiple endocrine neoplasia-1 (MEN-1) for non-invasive tumors [Herman *et al.*, 1993]. However, development of tumors from a specific gene mutation has not been definitely proven. Prolactinomas have been linked to estrogen exposure in humans and animals. The mechanism by which estrogen increases mitogenesis in lactotropes has been well studied and is the primary focus of this section.

Pituitary tumors in experimental animals can be induced by estrogen. Most of these estrogen-induced pituitary tumors are PRL- or GH-secreting tumors [Sadoul *et al.*, 1992; Ishibashi and Yamaji, 1985]. In both sexes of rats, long-term elevation of serum estrogen causes hyperplasia and/or adenomas [DeNicola *et al.*, 1978; Wickland *et al.*, 1981; Sarkar *et al.*, 1983]. In Fischer-344 female rats maintenance of constant, elevated, systemic estradiol levels by subcutaneous implantation of 17 $\beta$ -estradiol-containing silastic capsules induces prolactinomas very rapidly; within 2 weeks of estrogen implantation [Wickland *et al.*, 1981; Sarkar *et al.*, 1982; Banerjee *et al.*, 1994]. Subcutaneous implantation of 17 $\beta$ -estradiol-containing silastic capsules induces prolactinomas in either ovary intact or ovariectomized ACI rats [Shull *et al.*, 1997]. Subcutaneous implantation of diethylstilbestrol (DES) causes pituitary hyperplasia and neoplasm in Fischer-344 rats [Lloyd, 1983]. In estrogen-sensitive rats, short-term administration of the steroid stimulates lactotropic cell proliferation and, in the long-term, treatment results in lactotropic cell hyperplasia.

Estrogen also appears to promote prolactinomas in humans. There are reports of the development of a prolactinoma in a male-to-female transsexual who received massive doses of estrogen [Gooren *et al.*, 1987]. Some men with prolactinomas showed increased serum estradiol levels due to aromatized testosterone to estradiol [Prior *et al.*, 1987]. There is evidence of growth of a microprolactinoma to a macroprolactinoma during estrogen therapy [Garcia and Kapcala, 1995]. Women taking oral estrogen contraceptive or hormonal replacement therapy showed higher prolactin levels [Carol *et al.*, 1988; Fahy *et al.*, 1992]. Women using oral contraceptive due to menstrual irregularities showed a 7- to 8-fold higher incidence of prolactinomas [Shy *et al.*, 1983]. Data suggest that some women are more sensitive to the lactogenic effects of exogenous estrogen and, therefore, may be at greater risk for developing prolactinomas [Luciano *et al.*, 1985]. During pregnancy, estrogen levels elevate and the number of PRL-secreting cells and serum prolactin content increases. These elevated levels of estrogen are associated with symptomatic pituitary tumor enlargement (>1 cm) in up to 30% of women with macroadenomas and may result in persistent hyperprolactinemia and postpartum amenorrhea or galactorrhea [Gomez *et al.*, 1977]. However, the risk for development of significant clinical symptoms related to tumor expansion is less than 2% in pregnant women with microadenomas [Tonner and Schlechte, 1993]. There appears to be a close association between the use of oral estrogenic contraceptives and the onset of amenorrhea often accompanied by galactorrhea [Gomez *et al.*, 1977; Sherman *et al.*, 1978; Chang *et al.*, 1977]. Hence, estrogen can be considered a risk factor for the development of prolactinomas in some laboratory animals and in a certain population of humans.

To understand how estrogen functions in cell proliferation and transformation, it is critical to understand the receptor mediation that occurs as a first step in the cascade that follows the

introduction of estrogen into the cell. Steroid receptors and their related proteins are involved in a number of biological processes including reproduction, cellular differentiation, development, and metabolism. The transcriptional effects of estrogens are mediated by two closely related receptor isoforms, ER $\alpha$  and ER $\beta$ , each of which is encoded by a separate gene [Green *et al.*, 1989; Kuiper, 1996; Mosselman *et al.*, 1996]. The classical estrogen receptor is a typical steroid receptor consisting of a DNA-binding region, a hormone-binding domain, and two transactivation regions. One transactivation region is in the amino terminal portion of the receptor; the other is in the “hinge” region between the hormone- and DNA-binding domains. The importance of the two transcriptional activation regions in the receptor depends on cell type and the target gene. In the absence of estrogen, the receptors associate with binding proteins. This most likely serves to prevent inactive receptors from binding DNA. It has been reported that there are nearly 20,000 receptors per target cell [Katzenellenbogen and Gorski, 1975], and many of these may be spare receptors. This appears to be of major significance in lactotropes. It was recently demonstrated that only a small pool of estrogen receptors is required to regulate growth of these cells, while PRL synthesis requires activation of a larger number of estrogen receptors [Chun *et al.*, 1998].

In the pituitary, ER $\alpha$  mRNA and protein have been demonstrated in normal and adenomatous lactotropes [Friend *et al.*, 1994]. In addition, tumor-specific splice variants of ER $\alpha$  mRNA have been characterized in human prolactinoma specimens [Chaidarun *et al.*, 1997]. The prolactin tumor tissue specimens also coexpressed ER $\beta$ , which has a high homology with ER $\alpha$  in the DNA- and ligand-binding domain, but encodes a distinct transcriptional activating function-1 (AF-1) domain [Enmark *et al.*, 1997]. Recently, functional interactions between these receptors and receptor variants have been studied *in vitro* [Chaidarun *et al.*, 1998]. It has been found that both ER $\alpha$  and ER $\beta$  isoforms mediate the mitogenic and hormonal regulatory effects of estradiol in prolactinomas. Additionally, tumor-specific alternative spliced ER $\alpha$  variants promote estradiol-regulated cell proliferation. Hence, coexpression and interaction of various ER isoforms may be important in the promotion of neoplastic lactotropic cell proliferation in the pituitary gland.

Steroids, acting through their receptors, can regulate the synthesis of growth factors and growth factor receptors [Lingham *et al.*, 1988; DiAugustine *et al.*, 1988]. There are a number of growth factors that are estrogen-dependent and function in lactotropic proliferation, differentiation, and/or transformation. The relatedness of these factors and the significance of each alone are not well understood. Some of these estrogen-regulated factors are epidermal growth factor (EGF), platelet-derived growth factor (PDGF), insulin-like growth factor-1 (IGF-1) and IGF-2, transforming growth factor alpha (TGF- $\alpha$ ), basic fibroblast growth factor (bFGF), interleukin-2 (IL-2) and IL-6, nerve growth factor (NGF), fibroblast growth factor-4 (FGF-4), and transforming growth factor- $\beta$  (TGF- $\beta$ ). In particular, EGF, TGF- $\alpha$ , PDGF, IGF-1 and IGF-2 have been shown to stimulate lactotropic cell growth, and the roles of these growth factors in estradiol mitogenic action on lactotropes have been reviewed [Sarkar *et al.*, 1998]. TGF- $\beta$ -related peptides show stimulatory and inhibitory actions on lactotropes; and the TGF- $\beta$  receptor knockout mice develop pituitary tumors [Hentges *et al.*, 2000b; Shida *et al.*, 1998]. Recently, pituitary tumor-transforming gene (PTTG1) has been isolated and shown to express in pituitary adenomas [Zhang *et al.*, 1999]. This PTTG1 peptide has been shown to induce bFGF production from lactotropes and to mediate estradiol action on bFGF [Heaney *et al.*, 1999]. It has been suggested that both PTTG1 and bFGF play key roles in pituitary tumorigenesis.

In addition to the growth factors, estrogen alters secretion of various hormones that regulate lactotropic cell function. Of these hormones, dopamine, galanin and PRL appear significant. Dopamine is a physiological regulator of PRL secretion. Estradiol administration reduces dopamine secretion, alters dopamine D2 receptor splicing in the pituitary [Ben-Jonathan and Hanasco, 2001] and causes a loss of dopamine neurons in the hypothalamus [Sarkar *et al.*,

1982]. In dopamine D2 receptor knockout mice, prolactinomas develop spontaneously [Asa *et al.*, 1999] suggesting that a loss of dopamine function promotes prolactinoma development and may be a part of estrogen tumorigenic action.

Prolactin may participate in dopamine-regulated prolactinomas, since either a lack of PRL receptor signaling or PRL gene-disruption in mice will result in reduced dopamine function and the development of prolactinomas [Schuff *et al.*, 2002; Cruz-Soto *et al.*, 2002]. Estrogen increases galanin production in lactotropes [Hsu *et al.*, 1990], and targeted overexpression of galanin in the lactotropes of transgenic mice induces hyperprolactinemia and pituitary growth [Cai *et al.*, 1999]. These data indicate that galanin production may also be critical during estrogen-induced pituitary tumorigenesis.

There is evidence suggesting that members of the steroid receptor family may be recipients and transducers of signals initiated at the cell membrane [Weigel, 1994]. Estrogen is known to stimulate the protein kinase C pathway and induce *fos* and *jun* expression. There are reports that stimulating constituents of the signaling pathway mimics the effects of estrogen without any of the hormone present [Beck *et al.*, 1992; Aronica and Katzenellenbogen, 1991]. Ligand-independent activation of ER by cAMP has been shown to result from direct PKA or MAPK phosphorylation of ER isoforms [El-Tanani and Green, 1997; Chen *et al.*, 1993]. A direct physical and functional interaction between Sma and MAD-related protein 3 (Smad3) and ER $\alpha$  by two-way crosstalk has been identified in which TGF- $\beta$  signaling is suppressed by ER whereas ER-mediated transcriptional activation is enhanced by TGF- $\beta$  signaling [Matsuda *et al.*, 2001]. It has been proposed that bone morphogenic proteins (BMPs) and other TGF- $\beta$  family peptides induce pituitary prolactinoma pathogenesis through Smad3 and ER receptor crosstalk [Páez-Pereda *et al.*, 2003]. This indicates that there is a great deal of interaction between the estrogen receptors and other signal transduction pathways.

Estrogen-induced prolactinomas may also involve the regulation of cell-cell communication between lactotropes and other pituitary cells critical for cell growth, differentiation, and survival. Folliculostellate (FS) cells are known to be the macrophages of the anterior pituitary (AP). Schechter *et al.* [1988] demonstrated that estradiol activates FS cells as phagocytes during the onset of prolactinoma formation. They demonstrated that the vascular reorganization that occurs in response to estradiol is dependent upon activation of FS cells. This raises the interesting possibility that FS cells may be key regulators in the development and maintenance of estradiol-induced prolactinomas. The involvement of FS cells in angiogenesis is consistent with the finding that bFGF, a potent angiogenic factor, is secreted in large quantities from FS cells [Ferrara *et al.*, 1987]. The production of bFGF is increased in response to estradiol [Baird *et al.*, 1986]. These data indicate that FS cells may mediate the angiogenesis that follows estradiol exposure. In fact, it has been determined that a significant difference exists in the distribution of FS cells in low estrogen-responsive Sprague-Dawley and high-responsive Fischer-344 rats [Schechter and Weiner, 1991]. This difference allows for angiogenesis to occur in the blood vessels in adjacent meninges, resulting in systemic blood supply to the pituitary. Due to the recruitment of an arterial blood supply, the pituitary can escape the inhibitory influences of the hypothalamus. Therefore, estradiol induces tumors not only by increasing vascularization to the pituitary in these animals, but also by exerting its mitogenic effects on lactotropes unopposed by the inhibitory actions of dopamine.

Current data indicate that bFGF not only functions in angiogenic events but also stimulates lactotropic cell proliferation [Hentges *et al.*, 2000b]. Of major interest is the finding that TGF- $\beta$ 3, produced by lactotropes, induces bFGF release [Hentges *et al.*, 2000a]. In addition to TGF- $\beta$ 3, lactotropes secrete TGF- $\beta$ 1 [Sarkar *et al.*, 1992]. This cytokine is a potent inhibitor of lactotropic proliferation and prolactin secretion both *in vivo* and *in vitro* [Hentges and Sarkar, 2001]. Inhibitory response to TGF- $\beta$ 1 is altered in AP cell lines, such as the PRL-secreting



PR1 line and the GH- and PRL-secreting GH<sub>3</sub> cell line. Both of these cell lines show low levels of TGF- $\beta$ 1 and its type II receptor, TGF- $\beta$ RII mRNA [Sarkar *et al.*, 1998]. It has also been shown that lactotropes, exposed to estradiol, express reduced levels of TGF- $\beta$ RII and TGF- $\beta$ 1 and it has been proposed that the steroid increases TGF- $\beta$ 3 production and secretion from lactotropes. The secreted TGF- $\beta$ 3 is transported to the neighboring FS cells where it acts to induce the release of bFGF. The FS cell-derived bFGF stimulates lactotropic cell proliferation as lactotropes escape the effects of TGF- $\beta$ 1 growth inhibition and are activated by estradiol to express FGF receptors. It is also postulated that the loss of TGF- $\beta$ 1 growth inhibitory control may be a contributing factor for lactotropic cell transformation [Hentges and Sarkar, 2001].

Current data indicate TGF- $\beta$  family related peptides play important role in normal and transformed lactotropic cell growth control by acting directly on the lactotropes and regulating cell-cell communication between FS cells and lactotropes critical for lactotropes' proliferation and neovascularization for tumor survival. This is of future clinical significance. If pituitary tumorigenesis is indeed a stepwise process as it appears to be, it would be feasible to target the later events like TGF- $\beta$  signaling. Indeed, TGF- $\beta$ s have long been examined for their clinical relevance. However, there has been limited success due to the many actions of these factors. The tissue-targeted gene delivery to modulate pituitary production or action of TGF- $\beta$  might prove to be beneficial for development of new gene therapy approaches for prolactinomas.

## EXPERIMENTAL GENE THERAPY IN PITUITARY TUMORS

Studies with adenoviral and herpes-derived vectors demonstrated that these two approaches could be used to efficiently transfer different types of genes into normal rat AP cells in primary culture as well as in the corticotropic AtT<sub>20</sub> and somatomammotropic GH<sub>3</sub> tumor cell lines [Castro *et al.*, 1997; Goya *et al.*, 1998]. Interestingly, neoplastic AP cells were found to be more susceptible to viral vector-mediated transduction than were normal AP cells. More recently, a herpes simplex virus type-1 (HSV1)-derived vector was shown to be highly effective *in vivo* for gene transfer in rat pituitary prolactinomas [Bolognani *et al.*, 2001].

A recombinant adenoviral (RAAd) vector, RAAdTK, harboring the HSV-1 thymidine kinase (TK) suicide gene under the control of the human cytomegalovirus (hCMV) promoter, was used to transfer the TK gene to GH<sub>3</sub> and AtT<sub>20</sub> cells. Incubation of RAAdTK-treated GH<sub>3</sub> and AtT<sub>20</sub> cells with the prodrug ganciclovir (which after phosphorylation by viral TK becomes toxic) caused ample destruction of the cultures [Windeatt *et al.*, 2000]. In the same study, estrogen/sulpiride-induced rat prolactinomas were stereotaxically injected with the same RAAdTK. Subsequent injection of the host animals with two daily i.p. doses of 25 mg ganciclovir/kg for 7 days succeeded in partially reducing AP tumor size and serum PRL levels. Gene therapy, using adenoviral vectors harboring the TK gene under the control of specific AP hormone promoters, namely the human GH and glycoprotein hormone  $\alpha$ -subunit promoters, was effective for the treatment of GH<sub>3</sub> and  $\alpha$ -subunit producing pituitary tumor cell lines *in vitro*, respectively [Lee *et al.*, 1999]. An adenoviral vector, encoding the TK gene under the control of the human PRL promoter was also effective to induce apoptosis in GH<sub>3</sub> cell cultures exposed to ganciclovir. It failed, however, to reduce the growth and PRL-secretory rate of estrogen-induced rat prolactinomas *in vivo* [Southgate *et al.*, 2000]. Adenoviral vectors, harboring the TK gene under both a promiscuous (hCMV) and the hPRL promoter, showed expression of the transgene for up to 3 months *in situ* in the normal rat anterior pituitary [Southgate *et al.*, 2001].

Nude mice carrying GH<sub>3</sub> cell-grafted subcutaneous tumors were effectively treated with an adenoviral vector harboring the TK gene under the control of the hGH promoter [Lee *et al.*, 1999]. More recently, a GH cell-type specific adenoviral vector was constructed in which a stuffer DNA fragment, flanked by two loxP sequences, was placed between the hGH promoter

and the diphtheria toxin gene (GHp-loxP-DT). When the GH-producing cell line GH<sub>4</sub> was co-transduced with this vector and with another one harboring the expression cassette for GHp-Cre, rapid destruction of the cultures occurred. Furthermore, subcutaneous GH<sub>4</sub> tumors, generated in nude mice, involuted rapidly when topically injected with the above vectors in the tumor area [Lee and Jameson, 2002]. *In vivo* studies in rats have shown that intravascular administration of adenoviral vectors; harboring the  $\beta$ -galactosidase reporter gene under the control of the hGH or the glycoprotein hormone  $\alpha$ -subunit promoters, fail to express the transgene at pituitary level. On the contrary, stereotaxic injection of these vectors into the pituitary of rats succeeded in selectively expressing the transgene in the appropriate AP cell populations [Lee *et al.*, 2000].

Direct stereotaxic injection into the sheep pituitary of Ad vectors carrying the  $\beta$ -galactosidase gene under the control of either the hPRL or hCMV promoters led to high levels of transgene expression up to 7 days after surgery (Davis *et al.*, 2002). Histologic examination of these pituitaries revealed varying degrees of inflammatory response, with periglandular fibrosis, lymphocytic infiltrate and venulitis in almost all cases.

Another type of gene therapy strategy for the treatment of pituitary cancer is that based on the transfer of a gene(s) with the ability to rescue the normal phenotype of tumor cells. This approach has been implemented in mice heterozygous for the retinoblastoma (RB) tumor suppressor gene (*Rb*<sup>+/-</sup> mice). Such mice develop and succumb to characteristic pituitary intermediate lobe melanotroph tumors [Hu *et al.*, 1994]. Transduction of tumor melanotrophic cells with a recombinant adenoviral vector (rAd5.R.Rb) carrying the human RB cDNA under the control of its own promoter, showed a high level of efficiency both *in vitro* and *in vivo* [Riley *et al.*, 1996]. Furthermore, intracranial delivery of this vector to mice carrying actively growing melanotrophic tumors significantly reduced tumor growth and prolonged animal survival. Melanotrophic tumor proliferative index and apoptotic rates were markedly lowered in the rAd5.R.Rb-treated animals, which also showed growth-inhibitory dopaminergic neuron reinnervation of melanotrophic cells. A point of concern regarding this study is that, although the authors claim an acceptable targeting of the mice's pituitary gland with their transauricular injection method, they intrapituitarily injected up to 20  $\mu$ l viral vector suspension per animal, a volume several-fold larger than that of a mouse pituitary gland [Riley *et al.*, 1996].

## REGULATABLE VECTOR SYSTEMS FOR THE TREATMENT OF PITUITARY TUMORS

Two central objectives in the development of viral vectors are the achievement of cell-type specificity for transgene delivery, and the design of vectors where, once the transgene is incorporated into the target cell, its expression can be regulated by small molecules. Cell-type specificity has already been achieved in pituitary tumor cells by placing transgenes under the control of cell-type specific promoters, like the GH,  $\alpha$ -glycoprotein and PRL promoters for pituitary tumor cells, as noted previously.

Early inducible gene expression systems encountered limitations, such as pleiotropic effects of the inducer, basal leakiness, toxicity of inducing agents and a low level of expression. Nevertheless, nontoxic, tightly-regulated control of transgene expression has been achieved with a tetracycline (Tet) gene control system [Gossen and Bujard, 1992]. Two types of Tet gene control system have been devised. A Tet-Off system is one such system. It is composed of a Tet transactivator (a hybrid protein termed tTA, which contains the Tet and DNA binding domains of the *E. coli* Tet repressor fused to VP16, a HSV1 transactivator), which binds to *E. coli*-derived Tet-resistance operon regulatory elements embedded within a minimal CMV promoter (tetO/minCMV). Binding of tTA to this region recruits strong cellular transcription factors thus, inducing transcription of a transgene inserted downstream. When Tet is present,

it binds to tTA causing the dissociation of tTA from the tetO/minCMV complex thereby, inhibiting transgene expression. The second type of gene control system is the Tet-On system. Here tTA is modified by four amino acids giving rise to the rtTA transactivator, which, by itself is inactive, but in the presence of the Tet analog doxycycline, binds to the tetO/minCMV region and induces high levels of transgene expression.

A number of Tet-regulatable viral systems have now been developed, including a Tet-off dual adenoviral system for the  $\beta$ -galactosidase reporter gene with the DNA sequence encoding the tTA transactivator placed under the control of the hPRL promoter. With this system, specific transgene expression in rodent lactotropic cells was achieved both *in vitro* and *in vivo*. Furthermore, this expression could be turned off by administration of doxycycline and then back on when the antibiotic was removed [Smith-Arica *et al.*, 2001]. A similar dual regulatable adenoviral vector system, in this case expressing tyrosine hydroxylase (TH) under the control of the hCMV promoter, has been also constructed. As TH is the rate-limiting enzyme for dopamine synthesis, the growth of both lactotropic cell lines (GH<sub>3</sub> and MMQ) and estrogen-induced rat prolactinomas was significantly inhibited when co-transduced with the two vector components of the regulatable TH vector system. The expression of TH could be turned off by doxycycline, and back on by the removal of the antibiotic [Williams *et al.*, 2001].

Future application of regulatable gene delivery systems to neuroendocrine models would be of enormous interest considering the highly-regulated nature of neuroendocrine function.

## POTENTIAL OF SUICIDE GENE THERAPY FOR THE TREATMENT OF HUMAN PITUITARY TUMORS

As indicated previously, the development of gene therapy approaches for the neuroendocrine system is still incipient. While some of these approaches have already generated a core of results that emerge as a promising area of research for the treatment of pituitary tumors, no clinical trials have yet been documented. We are aware of only one gene therapy study using human pituitary cells. In this study, primary cultures of human lactotroph adenoma cells were successfully transduced with an adenoviral vector harboring the cDNA for human TH. The transduced cells not only expressed immunocytochemically-detectable TH, but were also shown to release significant levels of L-DOPA and dopamine, which markedly reduced PRL release to the culture medium [Freese *et al.*, 1996].

As already indicated, pituitary tumors seldom generate distant metastases. Although this feature allows the surgeon to spare normal pituitary tissue without the risk of tumor cell dissemination, neoplastic cells infiltrating the normal pituitary parenchyma may give rise to tumor regrowths. As mentioned before, pituitary tumor surgery is usually complemented with radiotherapy but, although this therapeutic combination is often effective to prevent tumor regrowth, it usually causes hypopituitarism due to excessive destruction of the normal pituitary parenchyma [Littley *et al.*, 1991]. In this context, gene therapy appears to be a potentially superior alternative to radiotherapy for the treatment of pituitary tumors. In effect, after tumor removal, intrasurgical injection of an appropriate suicide vector into the spared pituitary parenchyma could achieve complete elimination of remaining tumor cells without affecting healthy cells (Fig. 1). This therapeutic combination for the treatment of pituitary tumors has already been proposed [Castro *et al.*, 2001].

As in other organs, adenoviral vectors have been shown to cause inflammation in the pituitary gland (see above), an adverse effect that makes it advisable to consider the use of less immunogenic viral vectors, like the adeno-associated viral vectors, for the treatment of pituitary tumors. It should also be pointed out that, although so far only the HSV1-TK-GCV suicide system has been used to implement destructive experimental gene therapy of pituitary tumors,



other available toxic systems might offer advantages over the TK-GCV system. Thus, studies with recombinant Ad vectors harboring cDNAs for three toxic genes, namely those encoding the HSV1-TK, the *E. coli* cytosine deaminase and the deoxycytidine kinase, showed that cytosine deaminase had the highest efficacy to reduce the growth of different human lung carcinoma cell lines (Hoganson *et al.*, 1996).

Antisense and dominant negative gene therapies may constitute alternative strategies for the treatment of pituitary tumors. Suppression of endogenous bFGF expression by means of an Ad vector carrying an antisense transgene for bFGF caused inhibition of the proliferation of human glioma cells. The same effect was achieved by means of an Ad vector carrying a transgene for a dominant negative FGF receptor (Aoki *et al.*, 2002).

## BEYOND GENE THERAPY: THE NANOTECHNOLOGICAL FRONTIER

The future of gene therapy is strongly linked to the evolution of gene transfer technology. The achievement of cell-type specificity for transgene delivery and regulatability in a single vector system is a reality that shows the impressive progress that viral vector technology is making. It should be pointed out, however, that despite ever-increasing refinements in gene vector technology, the progress in the therapeutic effectiveness of gene therapy may eventually reach a plateau. This is particularly true for the design of therapeutic strategies for chronic pathologies where a substantial number of important genes and gene networks are likely to deteriorate. It seems unlikely that exogenous gene transfer may be able to fully correct these multigenic derangements. At some point, genetic therapies will have to move from exogenous gene transfer (i.e. gene therapy) to endogenous gene repair. Certainly, the implementation of endogenous gene repair to cure or prevent complex pathologies like cancer will call for a quantum leap in technology, a transformation that observers from different venues of science and technology believe is about to happen; it is called the Nanotechnology Revolution [Drexler *et al.*, 1991].

Nanotechnology may be defined as a technology based on the precise manipulation of individual atoms and molecules in order to build complex structures, specified at the atomic level. Living systems function on the principles of nanotechnology, since they can build up structures as complex as a human being, assembling them atom by atom. Although nanotechnology is still in its infancy, there is evidence that the seminal achievements that will lead to a mature technology are already taking place [Macilwain, 2000; Nanotech, 2001]. Nanotechnology is already attracting interest from physicists, engineers, chemists and biologists, a growing number of whom are getting involved in nanotechnological R&D [National Nanotechnology Initiative, 2002]. There is also a growing number of biomedical researchers who believe that one of the contributions of nanotechnology will be the development of nanomedicine, a medical specialty based on the use of intelligent nanoinstruments (nanobots) to cure the organism “from within.” Although no nanomedical applications are yet available, theoretical articles and books on nanomedicine are already being actively generated [Freitas, 1999].

It seems possible, therefore, to envisage a future therapeutic scenario where treatment of malignant tumors can be effected by means of, for instance, a single i.v. administration of a few million self-replicating, self destruction-enabled nanobots. These nanobots would first replicate within the patient in order to attain an appropriate working number. They would then survey the whole organism, searching for and destroying or repairing atypical cells. Subsequently, self-destruction and non-toxic elimination of nanobots would take place. This might prove to be the most effective and physiological method for prevention and/or treatment of cancer and other cellular pathologies. Time will tell whether this vision will remain just that, or will instead become a 21<sup>st</sup>-century reality.

## Acknowledgments

The authors thank Ms. Yolanda Sosa for secretarial assistance with the manuscript and Ms. Stephanie Zimmerman for editorial assistance. This work was supported in part by grants to RGG from the Argentinian Research Council (CONICET) and the Argentinian Agency for the Promotion of Science and Technology (ANPCyT) to RG and NIH AA00220 to DKS.

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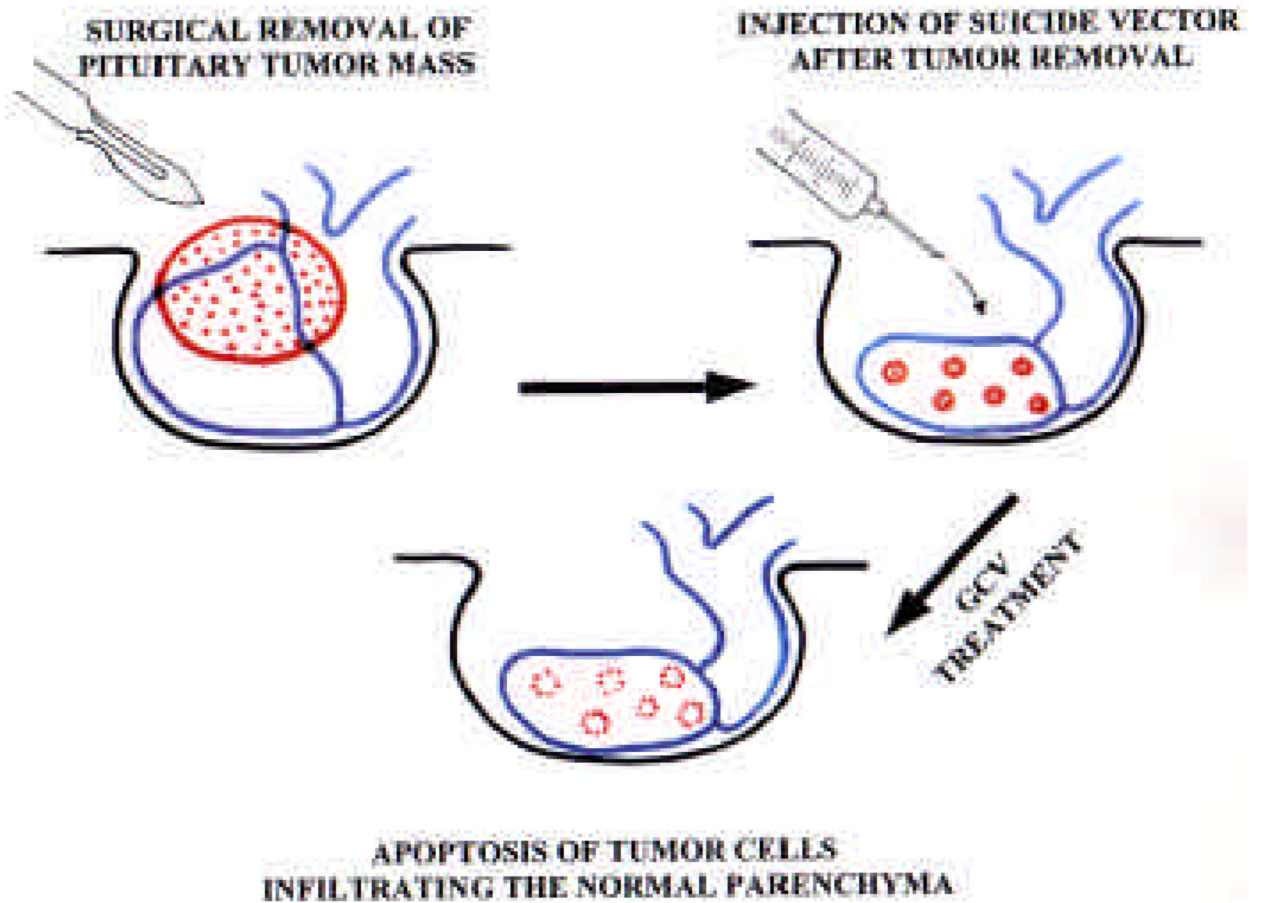
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**Fig. 1. Schematic representation of a possible application of suicide gene therapy to complement surgical treatment of pituitary tumors**

Initially the main tumor mass is removed, sparing the normal pituitary parenchyma. Subsequently, the surgeon injects in the pituitary tissue that surrounded the tumoral mass a suspension of an appropriate vector harboring the TK suicide transgene. A few days later the patient is treated with ganciclovir for a convenient period of time in order to eliminate those infiltrating tumor cells that escaped the scalpel.