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Morphologic Effects of hGRH Gene Expression on the Pituitary, Liver, and Pancreas of MT-hGRH Transgenic Mice

An *In Situ* Hybridization Analysis

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Morphologic changes in the pituitary, liver, and pancreas of mice with the metallothionein-human growth hormone–releasing hormone (MT-hGRH) transgene were analyzed by* in situ *hybridization histochemistry (ISH). There was progression from somatotroph hyperplasia to neoplasia in pituitaries of transgenic mice. Pituitary neoplasms were present between 9 to 12 months of age in some mice. Magnetic resonance imaging (MRI) readily identified enlarged pituitaries in MT-hGRH transgenic mice. Serum mouse GH and hGRH levels were markedly elevated in MT-hGRH transgenic mice.* In situ *hybridization histochemistry showed mRNA for hGRH in liver, pituitary, pancreas, spleen, and in most other tissues examined. Combined ISH and immunohistochemistry in the pituitary gland showed that some of the GH cells also produced hGRH, and ultrastructural immunohistochemical analysis of pituitaries showed that GH and hGRH were localized in the same cell and within the same secretory granules. Liver cells of MT-hGRH transgenic mice showed evidence of hypertrophy, and the pancreatic islets were hyperplastic with significant increases in the islet cell areas. The morphologic changes in the liver were distinctive enough to separate control littermates from MT-hGRH transgenic mice in all cases. The enlarged pancreatic islets had increased numbers of insulin-producing cells. Immunoreactive hGRH and hGRH mRNA were both localized in islet cells, and an intense hybridization signal for hGRH mRNA, but only weak staining for hGRH protein, were detected in the liver of transgenic mice. These results indicate that excessive hGRH production leads to distinct morphologic changes in various organs in MT-hGRH transgenic mice and that there is temporal progression from hyperplasia to adenomatous somatotrophs in pituitaries with chronic stimulation by hGRH that involves paracrine, endocrine, and autocrine mechanisms. (Am J Pathol 1992, 141:895–906)

The pathogenesis of pituitary neoplasia has not been clearly elucidated. The development of pituitary prolactin cell hyperplasia with progression to neoplasia after estrogen treatment has been studied extensively in rat models.[1,2] Transgenic mouse models in which a fusion gene encoding the promoter region of the mouse metallothionein-1 (MT) gene and the coding region of the human growth hormone–releasing hormone (hGRH) gene or the hGRH/mouse MT1/SV40 small t fusion gene have been used to study ectopic and eutopic expression of hGRH.[3–7] Several investigators have observed hyperplasia and adenomas developing in the pituitaries of these mice.[4,7] Immunohistochemical analysis of the hGRH transgenic mouse tissues has shown hGRH immunoreactive cells in the pituitary, pancreas, kidney, duodenum, lung, testes, and other organs, indicating that immunoreactive hGRH is produced and stored in these organs.[5] The use of *in situ* hybridization (ISH) with specific probes to detect hGRH mRNA along with immunostaining should allow analysis for the mRNA and protein in individual cells. Various investigators have analyzed the morphologic changes in mice with the ovine and bovine GH fu-

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sion genes and have found hypertrophy and other morphologic changes in the livers of these animals.[8,9] Although extensive studies have been performed on the localization of the protein hormone and the mRNA by Northern analysis in these mice, the morphologic changes resulting from high levels of hGRH and GH on tissues such as the pancreatic islets have not been previously examined.

In this report, we examined the expression of the hGRH transgene by ISH and the morphologic changes in the pituitary, liver, and pancreas in mice with the MT-hGRH transgene to study tissue- and cell-specific expression of hGRH and pituitary tumor development at the light microscopic and ultrastructural levels.

Materials and Methods

The MT-hGRH transgenic mice used in the current experiments were previously described.[3] Animals from the founder line 765-2 Tg(Mt-1, GHRF) Bri 11 were used for the present studies. Transgenic male mice with the MT-hGRH gene were originally identified by Southern blot hybridization of the hGRH cDNA probe to the DNA extracted from a portion of the tail. These transgenic males were mated with C57BL/6 × SJL/J females (Jackson Laboratory, Bar Harbor, ME). Transgenic progenies were identified by ISH using liver and other tissues to determine expression of hGRH and by radioimmunoassay (RIA) of serum extracts for hGRH.[10] Animals were weighed, killed by decapitation, and portions of tissues were quickly frozen in liquid nitrogen; other pieces were fixed in 4% paraformaldehyde pH 7.2 for 4 hours then embedded in paraffin. Serum was used for RIA of hGRH and mouse growth hormone (GH).

In Situ *Hybridization and Immunohistochemistry*

In situ hybridization histochemistry was done as previously described using paraffin-embedded and frozen tissue sections.[11,12] Hybridization was performed at 42°C for 18 to 24 hours using 1 to 2 × 10^6 cpm/slide of ^{35}S-labeled oligonucleotide probes followed by washing and autoradiography for 1 to 2 weeks then staining of the sections with hematoxylin and eosin (H&E). For combined ISH and immunocytochemistry, after overnight hybridization and washing in 2 × SSC (1 × SSC = 0.15 mol/l (molar) NaCl and 0.015 mol/l Na citrate), sections were stained with hGRH antiserum used at a 1/500 dilution.

The hGRH oligonucleotide probe was from exon 3, nucleotides 177–206 (5′-GTT GGT GAA GAT GGC ATC TGC ATA CCG CCG-3′) of the published hGRH cDNA.[13] This oligonucleotide recognized a single GRH mRNA species of approximately 750 nucleotides in mice with the hGRH transgene by Northern hybridization analysis. The rPRL and rGH probes were used as previously described.[11] Controls for ISH consisted of 1) treating tissues with RNAse before hybridization, which resulted in elimination of the hybridization signal, and 2) localizing hormones by immunochemistry in the same pituitary cells that expressed the mRNA.

Immunochemistry was performed as previously described.[11,12] Antibodies to rGH (used at a 1/10,000 dilution), rPRL (1/1000), ACTH (1/1000), rLHB (1/500), and TSHB (1/500) were from the National Pituitary Agency, Baltimore, Maryland. Antibodies to insulin (1/1000), glucagon (1/1000), somatostatin (1/1000), and pancreatic polypeptide (1/1000) were obtained from Dako Corp., Carpinteria, California. Anti-hGRH serum was used at a 1/500 dilution as previously reported.[5]

Northern Hybridization

Total RNA was extracted using the method of Chirgwin.[14] For Northern blot hybridization, 20-ug aliquots total RNA were denatured with formaldehyde and fractionated by electrophoresis on a 1% agarose gel containing 2 mol/l formaldehyde and transferred by blotting to a nylon membrane and hybridized with the ^{32}P-labeled hGRH oligonucleotide probe. Filters were washed and the bound probe was detected by autoradiography at −70°C using intensifying screens and different time exposures. Equal loading on the gel was ascertained by hybridization with a ^{32}P-labeled beta-actin oligonucleotide probe.[11]

Radioimmunoassay

Radioimmunoassay for serum GH and hGRH was performed as previously described.[5,6,10] The RIA for hGRH used an antibody that recognizes the midportion of the molecule.[10] The least detectable value for serum hGRH ranged from 1.6 to 3.2 ng/ml using 1 μl serum and for serum GH, 8 ng/ml using 10 μl serum.

Electron Microscopy

Sections of tissues were fixed in 2% formaldehyde in 2% phosphate-buffered glutaraldehyde in 0.1 mol/l cacodylate buffer pH 7.2, postfixed in 2% osmium tetroxide, and processed for electron microscopy. Ultrastructural immunochemistry was done as previously reported.[15] Briefly,

tissues were mounted on 300-mesh nickel grids, pretreated with a saturated aqueous solution of sodium metaperiodate, incubated with hGRH (1/1000), rPRL (1/1000) or rGH (1/10,000) antisera for 60 minutes, then incubated with 10 nm or 30 nm colloidal gold particles (Janssen Life Science Products, Amsterham Corp., IL) diluted 1:10 in phosphate-buffered saline for 30 minutes at room temperature. For double immunolabeling, the method of Bendayan was used[16] with labeling on separate sides of the nickel grid. The specificity of the immunostaining was checked by substituting normal rabbit serum for anti-rPRL, and anti-hGRH antisera and normal monkey serum for anti-GH antiserum. The rGH antiserum was treated with 10 μg/ml rPRL, and the rPRL antiserum was absorbed with 10 μg/ml rGH before use.

Morphometric Analysis

Morphometric analysis of islet cell area was done with a Bioquant IV instrument. A minimum of 10 islets were measured per case. The percentage of islet cells was determined by counting all of the cells stained for various hormones in a minimum of 20 islets. Results were expressed as the percentage of hormone producing cells per islet. All statistical analyses was done with Student's *t*-test.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) experiments were conducted on a Spectroscopy Imaging Systems Corporation MR system equipped with an Oxford Instruments 7 Tesla (300 MHz proton frequency) 18.3-cm horizontal bore superconducting magnet with actively shielded gradient coils. Sagittal mouse brain images were obtained using standard spin-echo MRI sequences and a two-dimensional Fourier transform image reconstruction technique. Four averages were acquired on a 128 × 256 matrix with a field of view of 3.0 cm, a slice thickness of 0.75 mm, a repetition time (TR) of 2.0 seconds, and an echo delay time (TE) of 60 milliseconds, which yielded T_2-weighted brain images.

For all MRI experiments, mice were anesthetized with 50 to 70 mg/kg sodium pentobarbital intraperitoneally and kept normothermic with a circulating warm water blanket during the imaging procedure.

Results

GRH mRNA Expression in Transgenic Mice

Northern hybridization analysis of liver tissues disclosed a 750-base mRNA species in mice with the hGRH transgene, but not in control littermates (Figure 1). Morphologic changes in the liver of mice with the hGRH transgene were distinctive with hypertrophied hepatocytes containing enlarged nuclei and prominent cytoplasmic invaginations into the nucleus (Figure 2). These invaginations were probably due to the marked cytoplasmic hypertrophy. *In situ* hybridization histochemistry analysis of various tissues detected GRH mRNA in most tissues examined, including liver, pancreas, pituitary, ovary, testes, spleen, kidney, and lung of transgenic, but not in control mice. The liver, exocrine pancreas, and pituitary had the most intense hybridization signal. Immunohistochemical analysis showed strong immunostaining for hGRH in the pituitary, and pancreatic islets, but very weak immunoreactivity in the liver and exocrine pancreas.

Morphologic Changes in the Pituitary

The body weight, pituitary weight, and serum GH and hGRH levels of transgenic animals were significantly increased compared with control littermates (Table 1). There was a significant increase in pituitary weight, which correlated with the age of the transgenic mice (Figure 3). Magnetic resonance imaging of the pituitary detected an enlarged gland in the MT-hGRH transgenic mouse com-

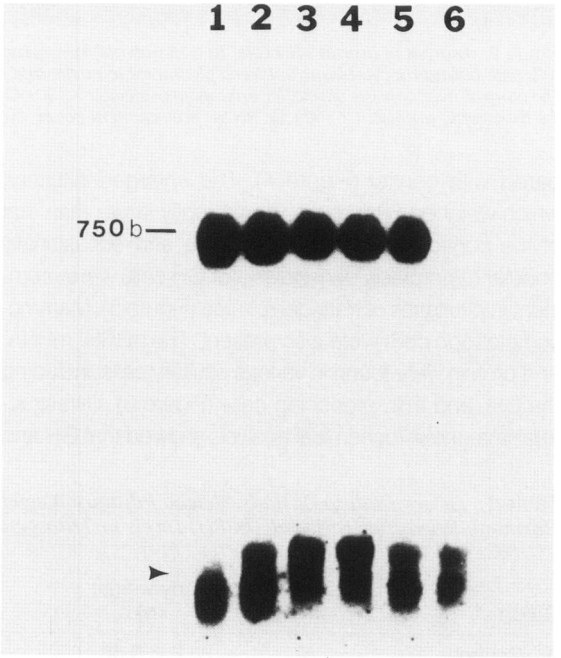

Figure 1. *Expression of hGRH in liver of MT-hGRH transgenic mice. Twenty micrograms of total liver RNA was fractioned on an agarose gel transferred to a nylon membrane and hybridized with a ^{32}P-labeled oligonucleotide hGRH probe (Upper panel). Lanes 1–5 are from MT-hGRH transgenic mice and lane 6 is from a control littermate. The same blot was used to hybridize with an actin oligonucleotide probe after washing off the hGRH probe and a 2.2 kb actin mRNA band was detected in each lane (lower panel).*

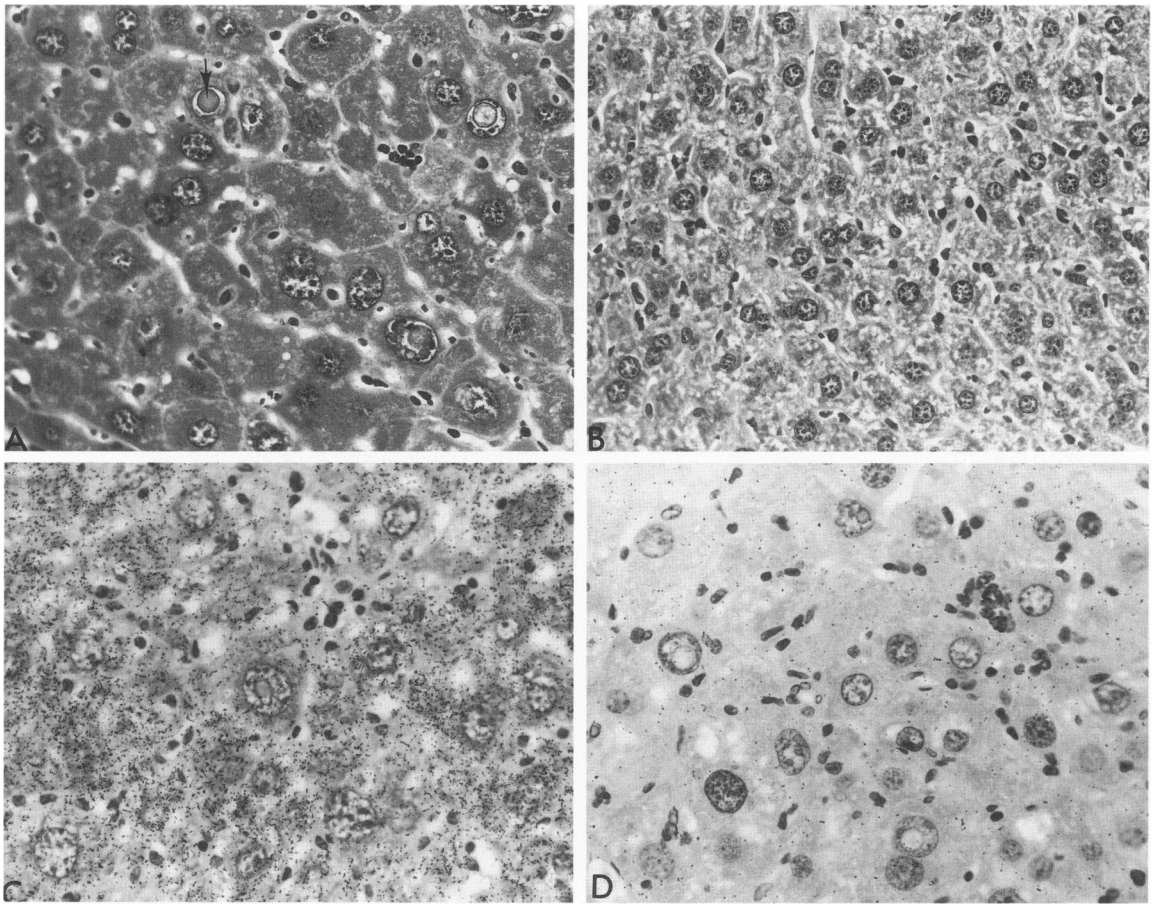

Figure 2. *Analysis of liver in MT-hGRH in 6-month-old transgenic and control mice.* **A:** *The liver in the transgenic mouse has hypertrophied cells with abundant cytoplasm, enlarged pleomorphic nuclei and prominent cytoplasmic invaginations into the nucleus (arrow) (×300).* **B:** *The control liver consists of smaller homogeneous cells (×300).* **C:** In situ *hybridization shows abundant hGRH expression by hepatocytes in the transgenic animals (×300).* **D:** *RNAse pretreatment before hybridization eliminated the positive signal (×300).*

pared with control (Figure 4). The enlarged pituitary, which weighed 53 mg, was significantly larger than that of the control gland by MRI. Diffuse and occasionally nodular hyperplasia or neoplasia of GH cells were prominent in pituitaries of transgenic mice (Figure 5). Mammosomatotroph cells were also present. The hGRH, mRNA, and protein were found in various pituitary cells, including the GH- and PRL-producing cells (Figure 6). Ultrastructural immunohistochemical analysis showed that GH and PRL were present in some of the same cells and in the same secretory granules (Figure 7). Both GH and hGRH were detected in the same secretory granules by ultrastructural immunohistochemical analyses (Figure 8).

Five pituitary lesions were classified as adenomas in mice in the 9- to 12-month group, and the other six glands in this group represented various stages of hyperplasia. Development of adenomas was supported by the following observations: 1) marked increase in the weight of the

Table 1. *Determination of Body Weight, Pituitary Weight, and Serum Growth Hormone (GH) and Human Growth Hormone Releasing Hormone (hGRH) Levels in Transgenic and Control Mice with Increasing Age*

Group	n	Body weight (g)	Pituitary weight (mg)	Serum GH (ng/ml)	Serum nGRH (ng/ml)
A—Control	5	25.8 ± 0.58	2.2 ± 0.2	25.8 ± 0.58	<1.6
A—Transgenic	6	40.6 ± 2.7***	19.6 ± 4.1**	6229 ± 917***	42.4 ± 4.3***
B—Control	5	25.8 ± 2.2	2.1 ± 0.12	—	—
B—Transgenic	4	44.2 ± 4.8**	37.1 ± 12*	3426 ± 1550	69.2 ± 25
C—Control	7	32.1 ± 0.8	2.0 ± 0.3	42.4 ± 11	6.0 ± 1.0
C—Transgenic	11	44.5 ± 1.6***	97.5 ± 25**	19,982 ± 6,704***	122 ± 23***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with control groups.
Group A = mice from 1 to 4 months of age; group B = mice from 5 to 8 months of age; group C = mice from 9 to 12 months of age.

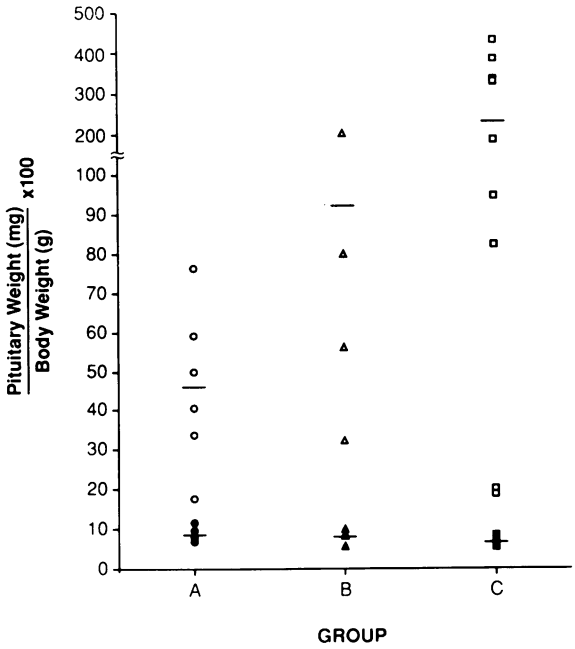

Figure 3. *Progressive enlargement of anterior pituitary gland with age in mice with the MT-hGH transgene. Group A, group B, and group C mice were 1–4 months, 5–8 months and 9–12 months of age, respectively. The closed circles, triangles, and squares represent control animals from Groups A, B and C. Group C animals with pituitaries greater than 100 mg or a (pituitary weight/body weight) (100) weight ratio greater than 300 had morphologic evidence of pituitary adenomas. The horizontal lines indicate the mean values.*

pituitary glands; the five mice with adenomas had pituitary weights of 139, 149, 150, 159, and 185 mg; 2) histologic examination of the pituitary showed monotonous sheets of chromophobic to slightly acidophilic cells containing large nuclei, prominent nucleoli (Figure 4), and occasional mitotic figures, and 3) immunohistochemical staining of adenomas showed that most adenomas were composed predominantly of GH-producing tumor cells with occasional PRL or thyroid-stimulating hormone (TSH)-positive cells. There were no adrenocorticotropic or gonadotroph cells. In contrast, hyperplastic pituitaries had various cell types admixed in the same area. Combined ISH and immunohistochemistry (IHC) analyses showed that mammosomatotropic cells containing both GH and PRL were relatively uncommon in areas of glands with adenomatous pituitaries, but were commonly found in hyperplastic pituitaries.

Most of the MT-hGRH transgenic mice studied were males, but similar changes were also seen in the pituitaries of female transgenic mice between 1 and 12 months of age in groups A, B, and C.

Morphologic Changes in Islets

The exocrine pancreas had a more intensely positive signal for hGRH mRNA than the pancreatic islets, but immunoreactive hGRH was found only within the islets (Figure 9). There was a greater than a twofold increase in the mean islet cell area in mice with the hGRH transgene compared with control mice (Table 2). Approximately 6% of the cells within the islets contained hGRH protein. Immunostaining of adjacent sections suggested that various cell types including somatotropic release inhibitory factor (SRIF) and glucagon cells also contained hGRH. There was a significant increase in the insulin- and pancreatic polypeptide-producing cells and a significant decrease in the percentage of glucagon-producing cells in the islets of hGRH transgenics compared with control mice (Table 2).

Serum glucose measured in four control and three MT-hGRH transgenic mice was 248 ± 30 and 151 ± 10 mg/dl, respectively ($P < 0.05$).

Discussion

Our study indicates that there is progression from normal to hyperplastic pituitaries and subsequent development of pituitary tumors in transgenic mice with the MT-hGRH gene and illustrates that this transformation can occur by the time the animals are 1 year of age. Recent studies by

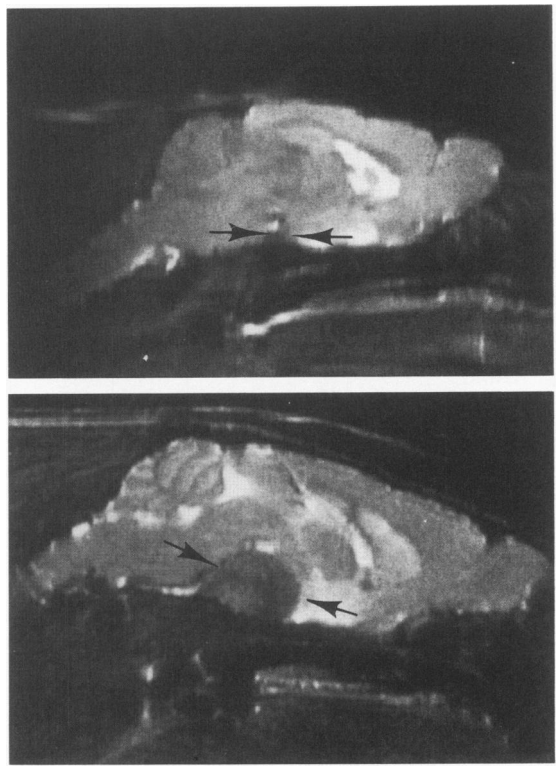

Figure 4. *T2-weighted mid-sagittal image of control* (A) *and transgenic MT-hGRH mice* (B) *at 6 months of age displaying a normal and an enlarged pituitary (arrows), respectively. The pituitary from the control mouse weighed 2 mg while that of the transgenic mouse weighed 53 mg. The bright area above the pituitary in* (A) *is due to the presence of cerebrospinal fluid in the ventricular system which is also evident above the enlarged pituitary in* (B).

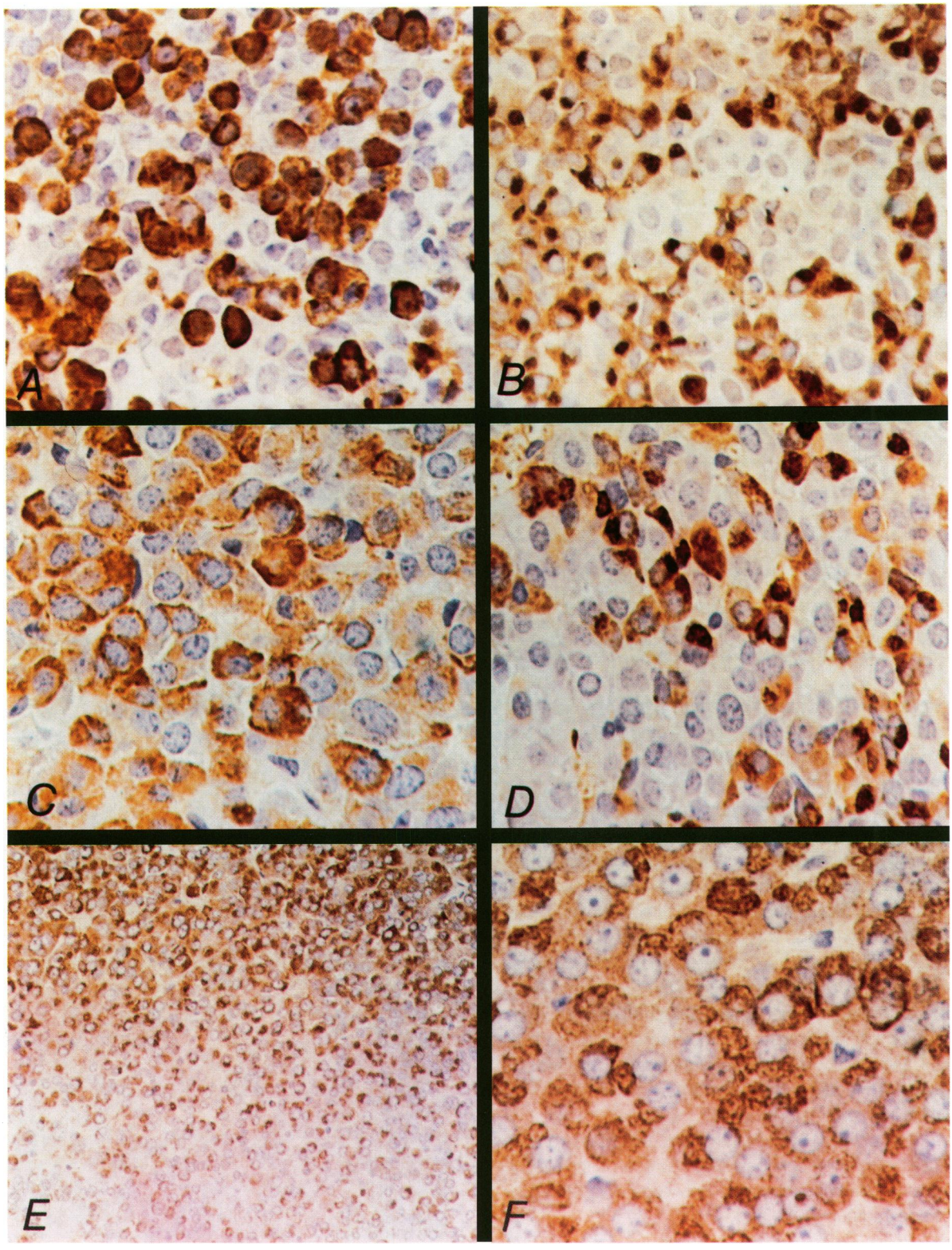

Figure 5. *Pituitary tissues from normal and MT-hGRH transgenic mice.* **A**: *Normal mouse pituitary stained for growth hormone (GH) (×300); and* **B**: *for prolactin (PRL (×300).* **C**: *Hyperplastic pituitary from a 4-month-old transgenic mouse stained for GH (×300).* **D**: *PRL showing enlarged cells with increased cytoplasmic and nuclear areas (×300).* **E**: *Adenomatous pituitary from a 10-month-old transgenic mouse showing hyperplastic GH cells in the bottom half and neoplastic GH cells in the top half of the photograph. The pituitary weighed more than 100 mg.* **F**: *Higher magnification showing the hyperplastic tumor cells with larger nuclei and more prominent nucleoli compared to the hyperplastic cells in C after GH immunostaining (×300).*

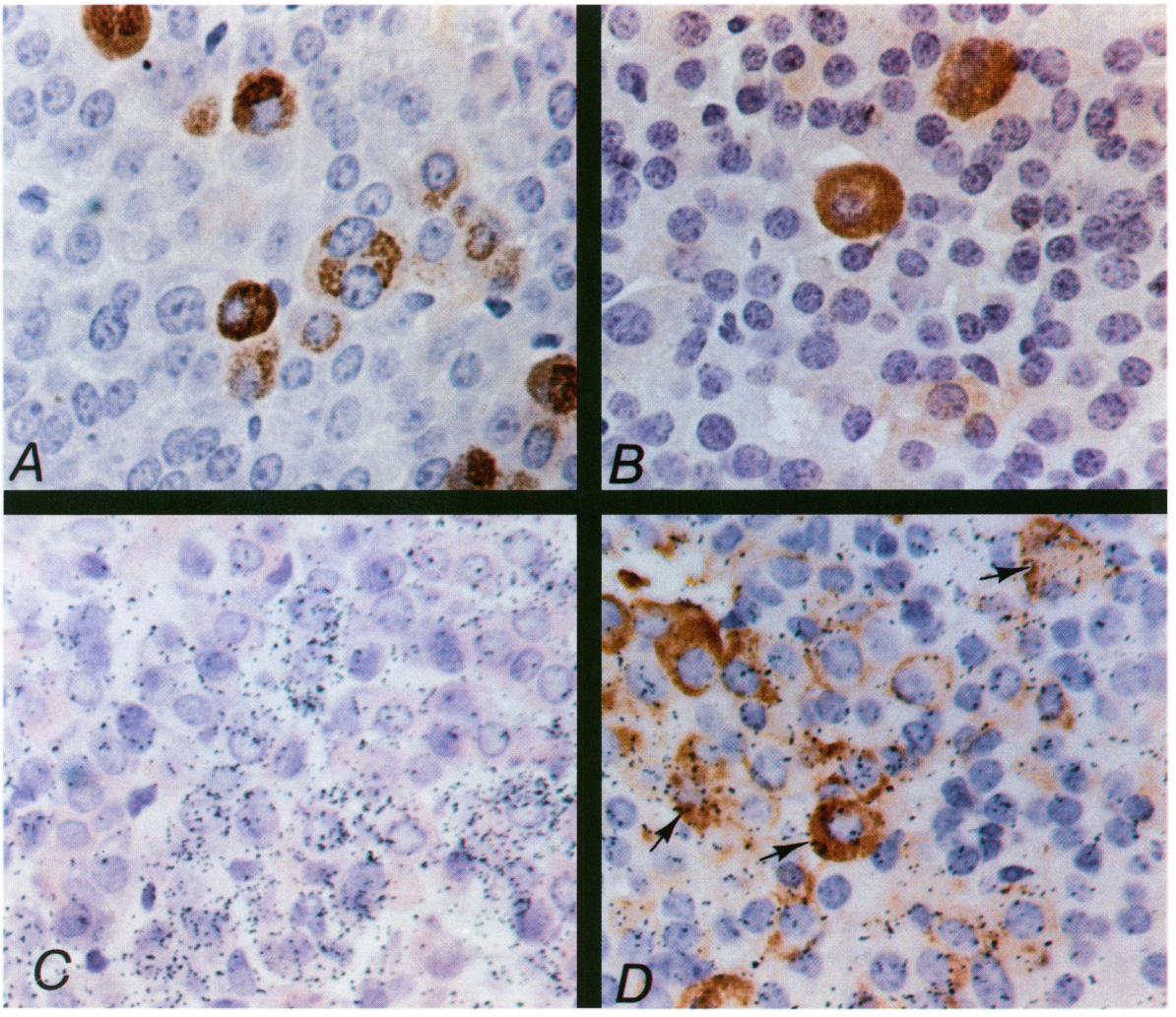

Figure 6. *Pituitary tissues from mice with the MT-hGRH transgene.* **A:** *Staining with PRL antiserum shows neoplastic PRL cells in this mixed GH-PRL adenoma (×300).* **B:** *Hyperplastic pituitary stained for hGRH showing positive immunoreactivity in a few cells (×300).* **C:** In situ *hybridization (ISH) localizing hGRH in many hyperplastic anterior pituitary cells (×300).* **D:** *Combined ISH with hGRH and immunostaining for GH indicating that GH cells (arrows) along with other cells express hGRH mRNA (×300).*

several investigators have shown that releasing hormones can act as specific growth factors. *In vitro* studies with rat pituitary cells by Billestrup et al[17] demonstrated that GRH stimulated GH cell proliferation in rat somatotroph cells and also induced *c-fos* expression.[18] Similarly, GRH infusion in normal rats led to enlargement of the pituitary within a few days with a 1.7-fold increase in pituitary weight.[19] Previous investigations with the MT-GRH transgenic mice showed significant pituitary hyperplasia in these animals.[4] In one mouse with the MT-GRF gene (animal 803–5), the pituitary weighted 132 mg at 2 years of age and was reported to show loss of normal morphology and was thought to be possibly neoplastic.[4] The studies of Asa et al.[7] showed that two transgenic mice with the hGRH/mouse metallothionein I/SV40 small t fusion gene had adenomatous pituitaries at 16 and 24 months of age.[7] In the current study, most of the adenoma cells were positive mainly for GH with occasional cells expressing also PRL or TSH. In contrast, many of the hyperplastic pituitary cells expressed both GH and PRL. The two adenomas described by Asa et al were both mammosomatotrophs, indicating that a spectrum of mixed or homogeneous GH or PRL tumors can be found in these mice.[7,20]

The use of MRI to visualize the pituitary gland in transgenic mice is an excellent method to study the progression of pituitary hyperplasia in a noninvasive way. This approach has been used to analyze changes in the pituitary of rats with PRL cell hyperplasia after estrogen treatment.[21,22] Because of the marked differences in weight of normal, hyperplastic, and adenomatous pituitaries in the current study, MRI can be used to follow the progressive enlargement of the gland in the same animal over an extended period.

The pathogenesis of adenoma development is not entirely clear. The presence of large amounts of hGRH in

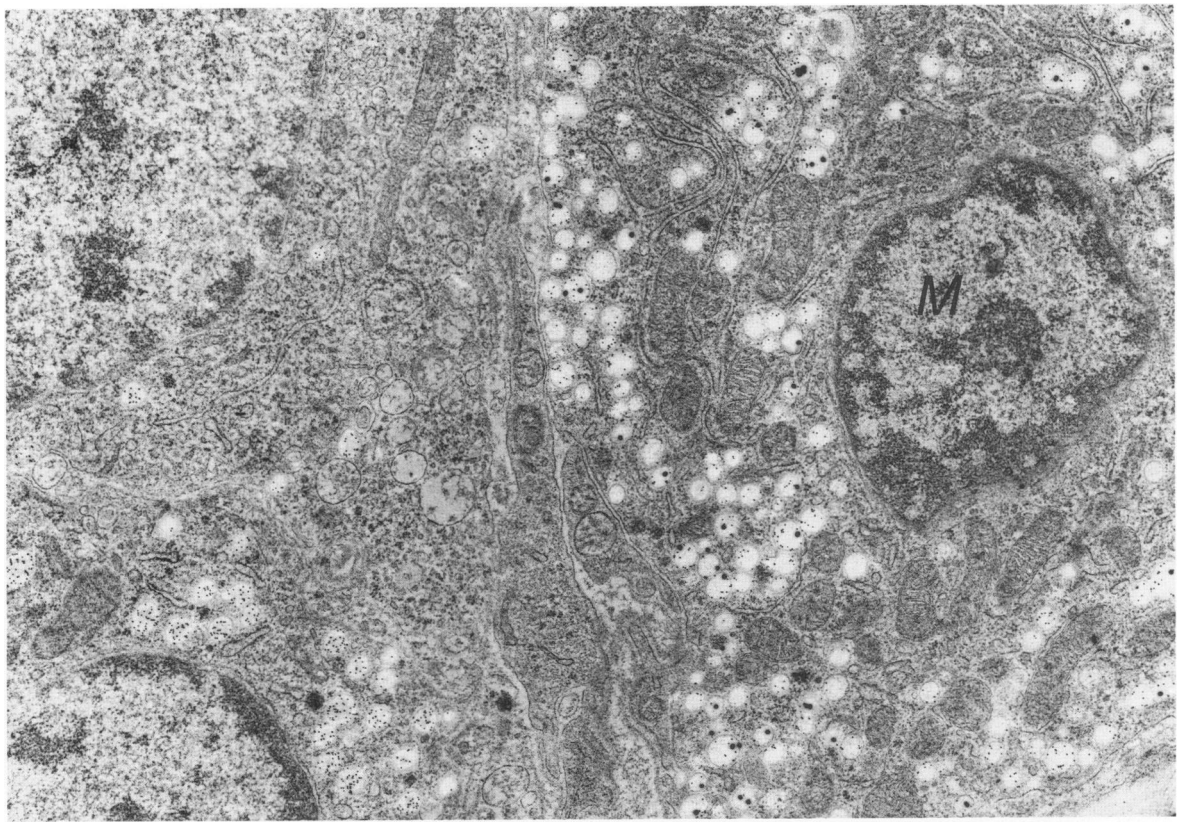

Figure 7. *Ultrastructural immunohistochemical analysis showing a mammosomatotropic cell (M) in a hyperplastic pituitary from an MT-hGRH transgenic mouse. The GH antigen is localized with the 10-nm gold particles and the PRL antigen with the 30-nm gold particles in some of the same secretory granules in the cell on the right. The cell on the lower left is positive only for GH (×20,000).*

the serum suggests that paracrine, endocrine, and autocrine stimulation by hGRH of the pituitary cells contributes to the hyperplasia to neoplasia sequence. The role of specific protooncogenes such as *c-fos* and possibly mutations of specific oncogenes and tumor suppressor genes in the progression from hyperplasia to neoplasia are largely unexplored. Other possible mechanisms that may contribute to neoplastic development in these hGRH transgenic mice including the effects of specific growth factors and specific transcription factors such as Pit-1/GHF-1[23–25] will have to be explored in future experiments.

Human growth hormone–releasing hormone was localized in GH and PRL cells in this study and ultrastructural immunochemistry showed that both GH and hGRH were in the same secretory granules. Recent studies by Frohman et al[6] with the MT-hGRH transgenic mice found that the highest levels of immunoreactive hGRH were in the pituitary, followed by the pancreas, with intermediate levels found in the liver and hypothalamus and lower levels in visceral organs, heart, and gonads. In previous studies of the pituitary, hGRH was present in GH cells as well as in PRL, gonadotroph, and thyrotroph cells at the light microscope level by some investigators[5] and in GH and thyrotroph cells, but not in PRL or gonadotroph cells by others.[4] These findings indicate that endocrine, paracrine, autocrine, and neuroendocrine secretion of hGRH could all contribute to the GH cell hyperplasia and neoplasia in hGRH transgenic mice.

The morphologic changes in the liver of mice with the hGRH transgene were very distinctive and correlated 100% with the expression of hGRH mRNA. Thus the morphologic appearance of the liver can be used to reliably identify mice with the MT-hGRH transgene. Similar findings in the liver in mice with the MT-oGH,[9] MT-bGH, and MT-GRF transgene have recently been reported to be secondary to hypertrophy and hyperplasia of hepatocytes with chronic elevations of serum GH levels.[8] The detection of abundant hGRH mRNA but lack of clearly identifiable cells containing the immunoreactive protein in the liver suggest that there was secretion of hGRH by the liver by the constitutive pathway. Because both endocrine and exocrine organs are processing hGRH, these tissues could be used as models to study intracellular trafficking and sorting mechanisms of this secretory protein by the constitutive and regulated pathways, as has been done with the GH transgenic model.[26]

Morphologic changes in the pancreatic islets were also distinct, with marked islet cell hyperplasia and increases in the percentage of immunoreactive insulin

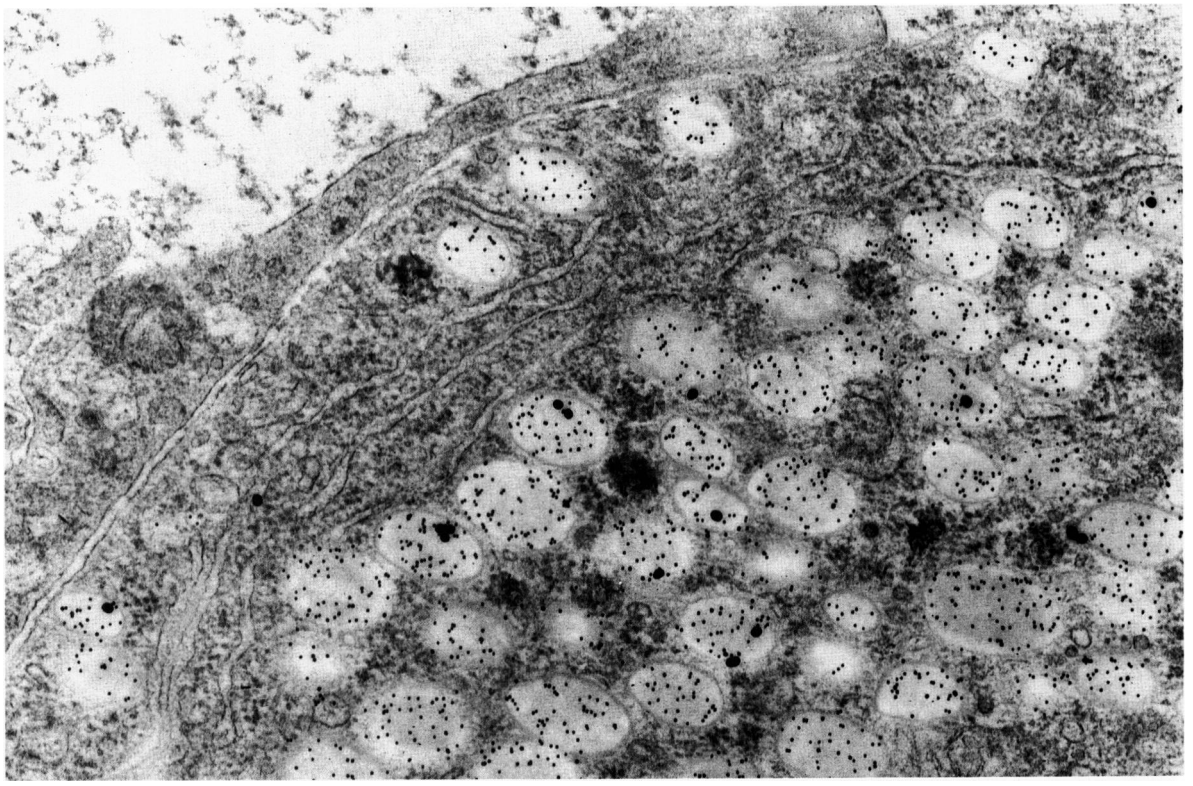

Figure 8. *Ultrastructural localization of GH with 10-nm gold particles and hGRH with 30-nm gold particles in the same secretory granules in a hyperplastic pituitary from a mouse with the MT-hGRH transgene. The rat GH antibody was made in monkey and the human GRH antibody was made in rabbit which avoids non-specific cross reaction. The localization of GH and hGRH in the same cell and in the same secretory granules suggests that there may be autocrine regulation of these cells by hGRH (x50,000).*

cells. Similar findings have not been reported previously in MT-hGRH transgenic mice, although they were not completely unexpected because of the known effects of excessive GH secretion on the pancreatic islets. The slightly lower serum levels of glucose in transgenic animals is similar to the observations of Quaife et al[8] in mice with the MT hGRF transgene. Interestingly these investigators also observed that the serum glucose levels in MT bGH mice were slightly elevated above control levels.[8] Our findings of islet cell hyperplasia and increased numbers of insulin-producing cells suggest that these effects are probably secondary to elevated serum GH levels with a compensatory increase in insulin-producing cells that may be seen in patients with acromegaly and severe insulin resistance.[27]

The studies of Furth and his colleagues[1,28,29] showed that pituitary tumors can develop in rodents after continuous direct stimulation by specific hormones such as estrogens or by perturbation of the normal feedback mechanism by endocrine ablation such as after thyroidectomy in mice. The question of when a hyperplastic pituitary transforms to a neoplasm has not been adequately addressed. Recent studies in rats treated with estrogen and dimethylbenz(a)anthracene showed that serial transplantation with estrogen supplements was needed to stimulate continued growth of hyperplastic pituitaries during transformation to tumors.[30] In the MT-hGRH transgenic mouse model, however, there is morphologic transformation from hyperplasia to adenomatous pituitaries in the same animals under the influence of GRH. *In vitro* studies and serial transplantation of the neoplastic cells in mice are required to analyze and to better understand the tumorigenic potential of the pituitary lesions in the MT-hGRH transgenic mice.

Acromegaly caused by GH-producing tumors is the second most common type of pituitary adenoma in humans.[31] The MT-hGRH model with progressive enlargement of the pituitary leading to development of pituitary adenomas has several similarities to human adenomas, including 1) human GH-producing adenomas are often mixed tumors with production of both PRL and GH[15,31,32]; 2) the ultrastructural and immunohistochemical features of human and hGRH transgenic mouse adenomas are similar; and 3) GRH is also produced by normal human pituitaries and by human GH adenomas[33] as well as by pituitary cells in the MT-hGRH transgenic mouse model. The most obvious difference between human GH-producing pituitary adenomas and the hGRH transgenic mouse model is that most human GH-producing adenomas are usually not associated with

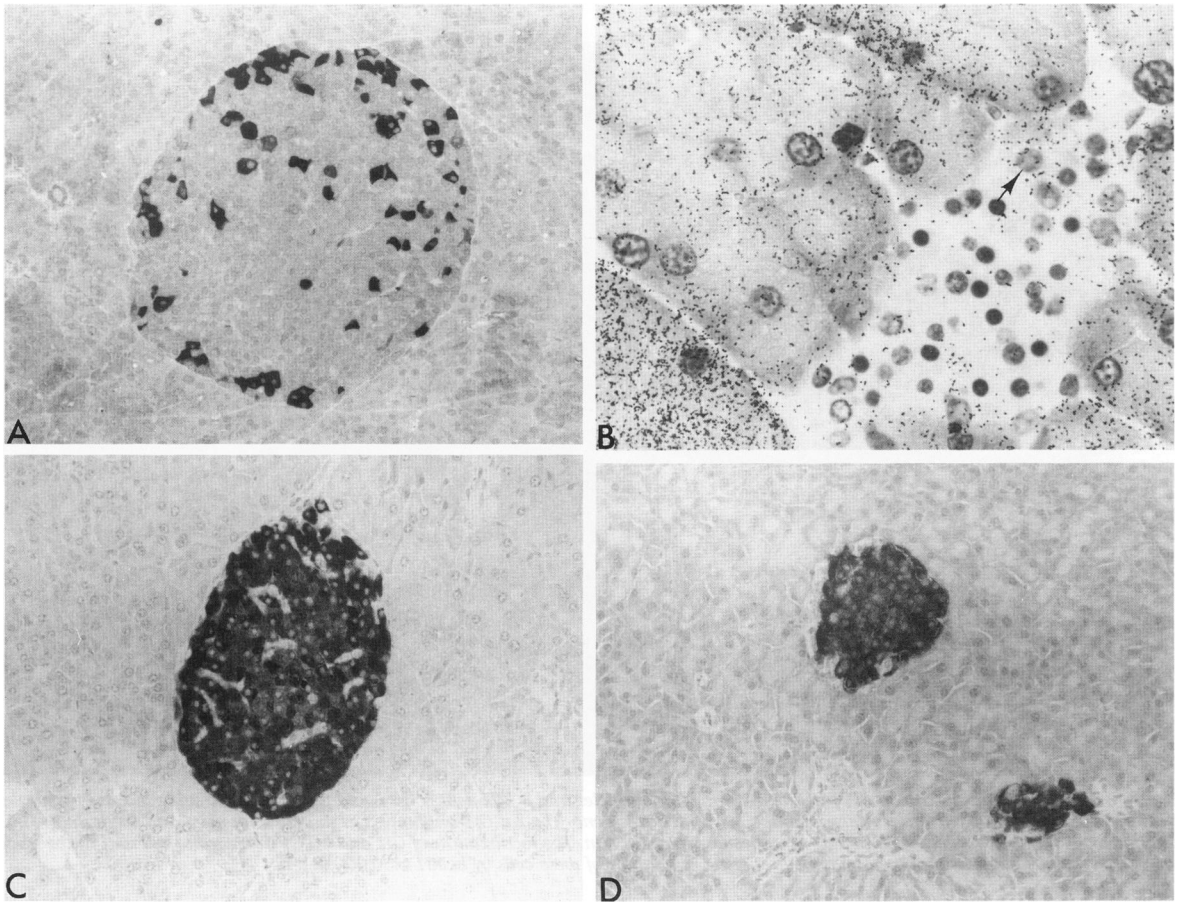

Figure 9. *Pancreatic tissues in MT-hGRH transgenic and control mice.* **A:** *Immunoreactive hGRH is prominent in the islet of a transgenic mouse (×210).* **B:** In situ *hybridization shows a strong hybridization signal for hGRH mRNA in the exocrine pancreas and focally positive cells in the islet (arrow) (×400).* **C:** *An enlarged islet with increased numbers of immunoreactive insulin cells are present in the pancreas from a transgenic mouse (×210).* **D:** *A control littermate had smaller islets with a lower percentage of insulin positive cells (×210).*

nodular and diffuse GH cell hyperplasia, as is seen in the transgenic mouse model. Nevertheless, ectopic production of hGRH has led to pituitary GH cell hyperplasia in humans,[34–36] and rare cases of GH-producing adenomas secondary to GRH producing hypothalamic gangliocytomas have also been reported.[37] In light of the numerous similarities between human adenomas and the transgenic mice, the analysis of the MT-hGRH transgenic mouse model may provide significant insights into the mechanisms regulating tumor development in human GH-secreting pituitary adenomas.

Note Added in Proof

Since this article was accepted for publication, we have established a cell line from the adenomatous pituitary tissues of an 11-month-old male transgenic mouse confirming that these enlarged pituitaries represent true adenomas.

Acknowledgments

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Table 2. *Morphometric Analysis of Areas and Immunoreactive Cells in Pancreatic Islets of Transgenic and Control Mice*

Group	n	Islet cell area $(\mu)^2$	Percent Immunoreactive Cell				
			hGRH	Insulin	Glucagon	SRIF	PP
Control	7	13,195 ± 3,003	6	73 ± 2	29 ± 2	16 ± 1	4 ± 1
Transgenic	8	32,733 ± 3384***	0	82 ± 1***	22 ± 1***	18 ± 1	8 ± 1**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with controls.
SRIF, somatostatin; PP, pancreatic polypeptide. Control and transgenic mice were between 4 to 10 months of age.

low for the pituitary antisera; and Drs. K. Mayo and R. Brinster for the parent MT-hGRH transgenic mice Tg (Mt-1, GHRF) Bri 11.

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