Chapter 2
Molecular Biology of Cushing's Disease

Ning-Ai Liu, Anat Ben-Shlomo, and Shlomo Melmed

Abstract The proximal molecular pathogenesis of ACTH-secreting pituitary adenomas remains enigmatic. Several transgenic mice models have contributed important knowledge to understanding human pituitary disease; animal and cell models have provided novel insights into mechanisms underlying the pathogenesis of ACTH-secreting pituitary adenomas, mostly due to cell cycle disruption. Defective glucocorticoid feedback mechanisms also likely lead to enhanced POMC expression and corticotroph proliferation. Novel peptide therapies targeting somatostatin and/or dopamine (D2) receptors may also provide further insights into ACTH-secreting pituitary tumor pathogenesis. Studies investigating microRNA expression in pituitary corticotroph adenomas point to important functions of a unique class of gene regulators in the molecular biology of Cushing's disease. Continuing research advancement will lead to better understanding of Cushing's disease and development of novel therapeutic approaches.

Keywords Corticotroph cell • Proopiomelanocortin (POMC) • Transgenic mouse models • CRH • Adrenocorticotropic hormone (ACTH)

S. Melmed (✉)
Cedars-Sinai Medical Center, 8700 Beverly Blvd., Room 2015, Los Angeles, CA 90048, USA
e-mail: melmed@csmc.edu

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Introduction

Despite advances leading to improved understanding of Cushing's disease, the pathogenesis of pituitary corticotroph adenomas remains enigmatic. We focus here on current knowledge and emphasize recent progress in identifying molecular and genetic mechanisms contributing to the development of pituitary corticotroph adenomas. Research progress on Cushing's disease pathogenesis is heavily dependent on animal studies largely due to the low disease incidence and small tumor size in humans.

Animal Models of Cushing's Disease and Related Tumors

Genetically manipulated mouse models have been used to recapitulate Cushing's disease, primarily because of striking homology in mammalian genomes as well as similar pituitary anatomy, cell biology, and physiology. Transgenic approaches have allowed overexpression of dominantly acting transgenes to phenocopy Cushing's disease pathology. Furthermore, specific allelic modification by homologous recombination gene ablation targeting endogenous cell cycle regulators have resulted in several mouse models with POMC-expressing tumors within the pituitary intermediate lobe.

Cushing's Disease Models with Transgenic Oncogene Overexpression

These models represent artificial phenomena generated using oncogenic viruses and, therefore, offer limited insight into corticotroph tumorigenesis. The first transgenic murine Cushing's disease model was produced by genetically introducing a hybrid gene consisting of the viral polyoma early region promoter linked to the polyoma large T antigen cDNA [1]. Transgenic mice developed pituitary microadenomas at 9 months of age, and large adenomas at 13–16 months of age, accompanied by features of Cushing's syndrome that progressed to wasting. The tumor latency period suggested the requirement for additional genetic or epigenetic alterations in pathogenesis of these tumors [1, 2]. Immunocompetent wild-type mice bearing transplants of PyLT transgenic pituitary tumors showed more pronounced effects of glucocorticoid excess than PyLT transgenic mice themselves. One of two PyLT transgenic lines developed pituitary tumors with 100% penetrance, suggesting that some viral oncogenes exhibit pituitary gland cell specificity.

Transgenic expression of the proopiomelanocortin (POMC) gene promoter (nucleotides −706 to +64) driving a simian virus (SV) 40 early gene encoding large T antigen induced large POMC-expressing pituitary tumors arising from the intermediate lobe [3]. Tumor cells expressed nuclear SV40 T antigen and POMC peptides, but not other pituitary hormones. Posttranslational pituitary

POMC processing was characterized by high proportions of acetylated and carboxyl-terminal shortened β-endorphins, as well as amino-terminal acetylated α-melanocyte-stimulating hormone, but virtually no ACTH(1–39), β-lipotropin or POMC. This pattern is indistinguishable from that of melanotrophs in the WT mouse intermediate lobe. In addition, tumor cells expressed abundant levels of mRNA for the prohormone convertase PC2 and undetectable levels of PC1, which is also similar to that of WT neurointermediate lobe, but distinct from the observed PC1 abundance in the anterior lobe.

Cushing's Disease Models with Transgenic Overexpression of Hormonal and Growth Factor Signals

Pituitary tumor growth appears to be promoted by hormones and growth factors implicated in normal pituitary function and development [6]. Mouse Cushing's disease models were developed by transgenic overexpression of hypothalamic stimulatory hormones or growth factors [4, 5]. Transgenic mice with metallothionein (mMT)-promoter-driven overexpression of CRH exhibited endocrine disruptions involving the hypothalamic-pituitary-adrenal (HPA) axis, manifesting as elevated plasma ACTH and glucocorticoid levels. These transgenic mice developed phenotypes similar to those seen in patients with Cushing's syndrome, such as excess fat accumulation, muscle atrophy, thin skin, and alopecia. However, there was no evidence of increased ACTH-expressing cells in the mMt-CRH transgenic pituitary, probably due to inhibitory feedback on pituitary corticotrophs by hypercortisolemia resulting from CRH stimulation [4].

Arginine-vasopressin is a potent ACTH-releasing hormone, which acts synergistically with CRH. Transgenic mice expressing the human V3 receptor under the control of rat POMC promoter sequences showed increased basal concentrations of corticosterone; however, no corticotroph tumors developed [7].

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine that regulates the HPA axis and enhances POMC transcription as well as ACTH secretion by potently synergizing with CRH [8]. LIF also regulates corticotroph cell proliferation [9]. Transgenic LIF overexpression targeted by the pituitary glycoprotein hormone α-subunit (αGSU) promoter lead to corticotroph hyperplasia, truncal obesity, thin skin, and hypercortisolism, all characteristic phenotypes of Cushing's disease. αGSU-LIF transgenic mice also exhibited central hypogonadism, dwarfism, and mild hypothyroidism, with gonadotroph, somatotroph, lactotroph, and thyrotroph hypoplasia. In the mouse, pituitary organ commitment is initiated with expression of alpha-GSU [5]. In the transgenic pituitary, LIF overexpression diverts progenitor cell differentiation from Lhx3/Lim3-dependent cell lineages (gonadotroph, thyrotroph, somatotroph, and lactotroph) to an Lhx3/Lim3-independent cell lineage, i.e., corticotrophs. Pituitary LIF signaling is further potentiated by glucocorticoids [10], therefore suggesting that neuro-immune-endocrine interfacing molecules act as important players in pituitary corticotroph homeostasis and tumor formation.

Table 2.1 Disrupted cell cycle regulators in mouse and human Cushing's disease and related tumors

Gene	Tumor-associated change	Tumor type	References
pRb	Mouse: heterozygous null mutation	IL tumors	[13]
p27	Mouse: null mutation	IL tumors in mouse	[15, 17]
	Human: reduced expression level a 19-bp duplication in exon 1	Corticotroph tumor and pituitary carcinoma	[44]
		MEN1-like syndrome including corticotroph tumor	[55, 56]
p18	Mouse: null mutation	IL and pituitary tumors	[20]
	Human: reduced expression level	Corticotroph adenomas	[48]
Cyclin E	Human: overexpression	Corticotroph adenomas	[47]
Pttg	Human: overexpression	All types of pituitary tumors including corticotroph adenomas	[42]

Genetic Knockout of Cell Cycle Regulators in Pituitary POMC-Cell Tumors

Multiple targeted gene knockout models have implicated cell cycle regulators in the pathogenesis of pituitary POMC-expressing tumors [11–13]. These gene knockout animals exhibit a high incidence of pituitary intermediate lobe POMC cell tumors, which are an otherwise rare tumor type in WT mice (Table 2.1). A classical example indicating the association of cell cycle regulators and pituitary tumorigenesis is derived from the heterozygous Rb mice [11–13]. The Rb gene encodes a tumor suppressor that controls the G1/S checkpoint. Rb phosphorylation by cyclin dependent kinases (Cdk) releases E2F, enabling S phase progression. Ink4-type inhibitors (p16, p15, p18, p19) and Cip/Kip-type (p21, p27, p57) suppress Cdk actions. Sequential activation and inactivation of protein kinase complexes regulate cell-cycle progression [14]. $Rb^{+/-}$ mice develop pituitary intermediate lobe POMC cell tumors at 12 months with 100% penetrance. p27 (Kip1) deletion, like deletion of the Rb gene, also leads to neoplastic growth within the intermediate lobe. However, intermediate lobe adenomas due to p27 deletion are less prominent than the POMC-expressing adenocarcinomas arising in $Rb^{+/-}$ animals [15–17]. Deletion of p27 or p21 in $Rb^{+/-}$ animals enhances intermediate lobe tumorigenesis and shortens the murine lifespan [18, 19]. Additionally, p18 deletion leads to intermediate lobe hyperplasia, which is further enhanced by compound loss of p27 or p21 [20, 21]. Overall, tumor incidence and phenotype are highly dependent on the mouse strain suggesting involvement of additional genetic factors in tumorigenesis [22]. Increased tumor incidence in $Rb^{+/-}$ mice is partially rescued by mutations of Rb effectors such as E2f1 or E2f4 [23, 24], as well as by pituitary tumor transforming gene (PTTG) [25]. PTTG is a securin that regulates sister-chromatid separation by binding to separase in the APC complex, and plays multiple roles in cell cycle regulation at different stages [26]. PTTG deletion

decreased pituitary tumor incidence in $Rb^{+/-}$ mice by triggering p53/p21-dependent senescence [27, 28]. Therefore, multiple cell cycle regulatory pathways are involved in initiating and maintaining pituitary corticotroph tumorigenesis.

Spontaneous Cushing's Disease in Large Animals

Spontaneous disorders mimicking human Cushing's disease have been described in dogs, horses, and less commonly cats [29–32]. Equine Cushing's disease usually results from intermediate lobe tumors, and rarely from those of the anterior lobe [29, 30]. Canine Cushing's disease has an estimated incidence of 1–2 cases/1,000 dogs/year [31, 32] and represents one of the most common endocrine disorders in dogs. Approximately 30% of canine Cushing's disease results from intermediate lobe tumors. In addition to typical melanotrophs, the canine pituitary intermediate lobe contains a substantial percentage of a second cell type that stains intensely for ACTH, but not for MSH [33]. Although molecular, cellular, and genetic makeup of canine corticotroph adenomas are yet to be identified, the high natural incidence and many clinical phenotypes similar to human Cushing's disease render canine Cushing's disease a potentially important system for both in vitro and in vivo studies to understand Cushing's disease pathogenesis, as well as to develop and test new therapeutic strategies.

Molecular Pathogenesis of Human Cushing's Disease

It remains unresolved whether corticotroph tumors arise from a primary defect in the hypothalamus or the pituitary [34]. However, currently, most evidence supports the primary pituitary origin of these tumors. Hypothalamic dysfunction was supported by the fact that many Cushing's disease associated endocrinopathies manifested as inhibition of growth, hypogonadotropic hypogonadism, and hypothyroidism. Moreover, in many cases the pituitary adenoma is not identified at surgery and these tumors often recur after apparently complete resection, while some pituitary glands harboring corticotroph adenomas exhibit corticotroph hyperplasia [35, 36]. However, corticotroph hyperplasia is difficult to detect as differences from normal corticotroph cells are subtle [37]. The evidence for a primary pituitary origin is more compelling. High cure rates with reversal of major abnormalities associated with Cushing's disease are observed after complete tumor resection and cortisol level normalization. Pituitary hyper-responsiveness to CRH before corticotroph adenoma removal reverses to hyporesponsiveness 1 week after resection [38]. Most corticotroph adenomas do not exhibit surrounding hyperplastic corticotrophs [37]. Moreover, pituitary tumors were proven to be monoclonal in origin [39, 40].

Biochemically and histologically, corticotroph tumor cells show relative and subtle abnormalities compared with normal ACTH-secreting cells, suggesting that tumorigenesis is likely associated with mutations or derangements of normal corticotroph-specific regulatory pathways. The initial event of corticotroph

transformation likely involves multifactorial etiologies such as genetic and epigenetic silencing of tumor suppressors, as well as hormonal and growth factor dysregulation, all of which may further promote tumor cell proliferation and expansion.

Tumor Suppressor Genes and Other Cell Cycle Regulators

Pituitary cells are rarely affected by oncogene activation or loss of tumor suppressor genes. Most protooncogene and tumor suppressor gene mutations implicated in nonpituitary cancers have not been identified in corticotroph adenomas. These include RAS, c-ERB2/neu, c-MYC, PKC, RET, c-MYB, c-FOS, Gα subunit of the G-protein, p53, Rb1, p16, and p18 [41].

As a cell cycle regulator and global transcription factor modulating G1/S and G2/M phase transition, human PTTG1 is overexpressed in more than 90% of all type of pituitary tumors, including corticotroph adenomas [42]. PTTG1 is regulated by CDK1-mediated phosphorylation [43], suggesting a link between cell cycle control by CDKs and PTTG1 function and implicating cell cycle deregulation in pituitary tumorigenesis. The p27 tumor suppressor regulates cell cycle progression by interacting with and inhibiting cyclin/Cdk complexes. Although early studies detected no p27 genomic mutations or consistent change in p27 messenger RNA expression in human sporadic pituitary tumors, downregulation of p27 protein expression is often observed in corticotroph adenomas and pituitary carcinomas suggesting underlying mechanisms involving posttranslational dysregulation [44]. Degradation of p27 is a critical event for the G1/S transition and occurs through ubiquitination by SCF(Skp2) and subsequent degradation by the 26S-proteasome [45]. In a study of 59 human pituitary samples (seven normal pituitary glands, 52 adenomas including 12 ACTH-secreting tumors), no significant difference of Skp2 mRNA or nuclear protein expression was detected between the normal pituitary and tumor tissue; therefore, it is not yet clear whether SKP2 is the relevant F-box protein for degradation of p27Kip1 in corticotropinomas [46]. In addition, increased cyclin E protein expression is frequently observed in corticotroph tumors, probably in relation to the low p27 protein expression levels [47]. Using Affymetrix GeneChip microarray analysis combined with RT-PCR analysis for gene expression profile of major pituitary adenoma subtypes, ACTH-secreting adenomas ($n = 13$) were shown to exhibit significantly underexpressed p18, in which murine gene deletion has been shown to produce pituitary ACTH cell hyperplasia and adenomas [48]. Both p27 and p18 are directly regulated by MEN1 (multiple endocrine neoplasia type 1), and loss of MEN1 function results in downregulation of these two inhibitors with subsequent deregulation in cell proliferation [49, 50]. The multiple endocrine neoplasia syndrome is characterized by predisposition to pituitary adenomas, parathyroid hyperplasia, and pancreatic endocrine tumors. Pituitary adenomas affect between 25 and 30% of MEN-1 patients [51]. According to the France–Belgium MEN1 multicenter study, 6 of 136 cases of MEN1 with pituitary adenomas harbored

ACTH-secreting corticotroph adenomas [52]. However, expression of MEN1 mRNA is normal in sporadic pituitary corticotroph adenomas [53, 54]. Recently, the CDKN1B/p27^{Kip1} gene has been identified as a new susceptibility gene for a MEN1-like syndrome that is MEN1-gene mutation negative (now designated MEN4), in one family segregating endocrine neoplasia (pituitary adenoma, acromegaly, and primary hyperparathyroidism) [55]. Subsequently, a second germ-line CDKN1B/p27^{Kip1} mutation was identified in 1 of 36 (2.8%) Dutch patients clinically suspected for MEN1, however, tested negative for *MEN1* gene mutation [56]. A 19-bp duplication within CDKN1B/p27^{Kip1} exon 1 changes the amino-acid sequence after 26 residues and leads to a premature stop codon 69 amino acids earlier than the wild type. The patient was diagnosed with small-cell neuroendocrine cervical carcinoma, ACTH-secreting pituitary adenoma, and hyperparathyroidism, all lesions compatible with MEN1 [56]. Overall, somatic CDKN1B/p27^{Kip1} mutations are uncommon in suspected MEN1 cases and sporadic pituitary adenoma patients [56–58] (Table 2.1).

Neuroendocrine Hormones and Regulatory Factors

Corticotroph proliferation and ACTH secretion are controlled by stimulatory factors, such as CRH, vasopressin, leukemia inhibitory factor (LIF), and inhibitory factors, such as glucocorticoid and somatostatin (SRIF), as well as their specific receptors. Genes encoding proteins involved in corticotroph regulatory pathways are potential candidates as tumorigenic mutations in Cushing's disease. However, studies investigating classic corticotroph regulatory factors are yet to provide clear evidence of a common genetic defect in these tumors.

CRH is the main hypothalamic stimulator of corticotroph proliferation and ACTH secretion. In humans with CRH-secreting tumors, excess CRH induces corticotroph hyperplasia and hypercortisolism but no corticotroph tumor formation [59, 60]. In a study of 43 corticotroph adenomas, CRH mRNA levels were significantly higher in tumor tissues vs. normal pituitary and also in macroadenoma and locally invasive adenomas vs. microadenomas. CRH expression correlated with Ki-67 expression, suggesting CRH autocrine/paracrine functions in corticotroph adenomas [61]. Some corticotroph adenoma cells exhibit increased CRH receptor type 1 mRNA levels; however, mutations of CRH receptor coding sequence have not been found [62]. Vasopressin type 3 receptor (V_3R) stimulation enhances ACTH secretion and mRNA expression is increased in ACTH-secreting tumors, probably as a consequence of chronic glucocorticoid exposure. However, no mutation in the V_3R gene has been found in corticotroph adenomas [63]. While the pathophysiological significance of V_3R and CRH/CRH-R overexpression in Cushing's disease remains to be determined, they may be associated with proproliferative effects sustaining corticotroph tumor growth.

One of the hallmarks of corticotroph adenomas is partial resistance to corticosteroid feedback, which may represent an early event of corticotroph tumorigenesis.

Corticotroph tumors likely develop from cells with genetic mutations rendering partial resistance to the physiological negative feedback [64], therefore leading to a set-point defect and inappropriately high ACTH levels. Peritumoral normal corticotrophs would likely exhibit growth suppression in response to the supraphysiological level of cortisol, thus providing the mutant clone with a further growth advantage. ACTH may suppress its own secretion from corticotrophs via an ultrashort paracrine/autocrine loop. Indeed, ACTH receptor and melanocortin 2 receptor (MC2) mRNAs were absent in 16 of 22 pituitary corticotroph adenomas, but were detectable in normal human pituitary. Plasma ACTH levels were significantly higher with tumors that did not express the receptor compared to those that did [65]. Loss of normal ACTH receptor expression and/or function in corticotroph adenomas may contribute to partial corticosteroid resistance, although no mutations of ACTH and MC2 receptors were found in corticotroph tumors that still exhibit receptor expression. Glucocorticoid exerts feedback on corticotrophs via the glucocorticoid receptor (GR), and GR disruption may contribute to pituitary-specific glucocorticoid resistance seen in corticotroph adenomas. The human GR exhibits two isoforms resulting from alternative transcript splicing [66]. GR-β differs from GR-α at the carboxyl terminus, which prevents corticosteroid binding and transcriptional activation [66]. A nonsense mutation leading to a truncated GR was discovered in a patient with Nelson's syndrome; however, no similar defect was identified in a series of 19 ACTH-secreting tumors, including two cases of Nelson's syndrome, three ectopic secretors, and one malignant corticotropinoma [67]. While a GR gene mutation does not appear to be a common defect contributing to glucocorticoid resistance in corticotroph adenomas, it remains to be determined whether GR LOH, or altered levels of GR-α and GR-β isoform expression are associated with Cushing's disease pathogenesis.

Investigation of mechanisms underlying glucocorticoid resistance has led to identification of two essential proteins for repression of proopiomelanocortin (POMC), a precursor of ACTH. Corticosteroids repress POMC transcription through protein–protein interactions of GR with NGFI-B to form a transrepression complex at the POMC promoter. The ATPase subunit of the chromatin remodeling Swi/Snf complex Brg1 is essential to stabilize GR and NGFI-B interactions, and critical for recruitment of the histone deacetylase HDAC2 to the complex [68]. In a series of 36 human corticotroph adenomas obtained at surgery, 50% of tumors were deficient in nuclear Brg1 or HDAC2. Brg1 was delocalized to the cytoplasm in a subset of tumors, while it was detected in nuclei of surrounding peritumoral corticotroph cells. This observation was apparent in both human and canine pituitary corticotroph adenoma cells [68, 69]. The relative high frequency of Brg1 and/or HDAC2 misexpression in corticotroph adenomas supports their importance in pituitary corticosteroid resistance associated with Cushing's disease.

Pituitary Nelson's tumors arise in patients with Cushing's disease who have undergone bilateral adrenalectomy. The cause for growth of Nelson's tumor is yet unknown, and recent studies suggest that tumors do not appear de novo, but rather grow from a persistent pituitary corticotroph microadenoma [70]. Potential causes of

Nelson's tumors may include restored CRH and AVP tone, elimination of the suppressive growth effect of endogenous cortisol and insufficient levels of exogenous cortisone [71]. Although usually slow growing, some tumors can grow rapidly to a large size [72]. Crooke hyalinization is usually absent in nontumorous corticotroph cells derived from pituitary glands harboring Nelson's tumors.

Corticotrophs are also negatively regulated by somatostatin (SRIF) signaling pathways. Somatostatin actions are mediated through five different membrane-bound receptors (SSTR 1–5). SSTRs are members of the G protein-coupled receptor family. SSTR signaling leads to inhibition of hormone secretion and cell proliferation, or may induce apoptosis. Human corticotroph adenomas exhibit abundant SSTR5, in addition to SSTR1, -2, and -3, mRNA and protein levels. Pasireotide (SOM230), a synthetic SRIF analog, inhibits ACTH secretion from ACTH-secreting adenomas not responsive to octreotide in vitro and is more effective than octreotide to inhibit CRH-induced rat ACTH and cortisol secretion. In a proof-of-concept, open-label, 15-day phase II trial, 76% of patients with Cushing's disease receiving pasireotide exhibited lowered urinary free cortisol levels [73]. Enhanced pasireotide action in corticotrophs is determined by SST5 dominance that maximally stimulates short- and long-term corticotroph responses to SRIF analogs [74].

In addition to the aforementioned hormonal and regulatory factors, other cytokines, growth and developmental factors have been investigated for potential roles in corticotroph tumor formation, including epidermal growth factor (EGF) and receptor (EGFR), PTX family members and Tpit/Tbx19 [41, 69], none of which has been found to play a major role in corticotroph tumorigenesis. These factors may regulate a preexisting tumor clone or promote establishment of an oncogenic background, therefore contributing to tumor formation and/or expansion. A mutation in the DAX1 gene that controls HPA axis development was found in a 33-year-old patient with X-linked adrenal hypoplasia congenita and pituitary corticotroph adenoma [75]. Recently, pituitary corticotroph microadenomas have been reported in two patients with tuberous sclerosis complex, an autosomal dominant neurocutaneous disorder characterized by benign tumors (hamartomas), epilepsy, and mental retardation. This complex is a result of mutation in the *TSC1* and *TSC2* genes that encode the proteins hamartin and tuberin, respectively. Mechanisms promoting corticotroph adenoma growth in this disorder are unknown [76].

MicroRNA Expression in Corticotroph Adenomas

MicroRNAs (miRNAs) are noncoding, single-stranded RNAs constituting a novel class of gene regulators. MicroRNAs control diverse biological processes including cell growth, differentiation and apoptosis by posttranscriptional regulation of target gene expression [77]. More than 50% of identified human microRNAs are located in the fragile sites of genome areas [78]. miRNA mutations or misexpression correlate with several human cancers suggesting that miRNAs can function as

tumor suppressors [79]. In a recent study of 11 ACTH-secreting pituitary adenomas and seven normal pituitaries, real-time PCR analysis revealed downregulation of several miRNAs in corticotroph adenomas compared with normal pituitary, including miR-15a, miR-16, and Let-7a among others [80]. Reduced miR-15a and miR-16 expression was also discovered in GH- or PRL-secreting pituitary adenomas, and levels of reduction correlated inversely with tumor diameter [81, 82]. Interestingly, miR-15a and miR-16 genes are colocalized with the Rb tumor suppressor on chromosome region 13q14, which is frequently deleted in pituitary adenomas including corticotropinomas [83, 84]. There has been evidence that additional putative tumor-suppressor gene(s) at the 13q14 locus are closely linked to, but distinct from, Rb1 and might be important in pituitary tumorigenesis [85]. Let-7 microRNA negatively regulates high-mobility group A2 (HMGA2), an embryonic and oncogenic protein that is highly expressed in many tumors including pituitary adenomas [86–88]. In a series of 55 postsurgical pituitary adenomas, decreased let-7 expression was present in 23 of 55 (42%) adenomas, including 12 of 18 (67%) corticotroph adenomas, and correlated with high-grade tumors ($P<0.05$). An inverse correlation between let-7 and high-mobility group A2 expression was evident ($R=-0.33$, $P<0.05$) [89]. These findings support a causal link between let-7 and HMGA2 whereby loss of let-7 expression induces HMGA2, contributing to pituitary tumorigenesis and progression.

Conclusion

In summary, human corticotroph tumor studies are difficult to undertake as the disease is rare. Moreover, these tumors are small, and in many cases the tumor specimen is accompanied by surrounding normal pituitary tissue. In addition, direct comparison of tumorous to normal corticotroph cell function is challenging in most cases, as normal pituitary tissue from the same patient is usually unavailable, and even if available, the degree of "normalcy" is questionable. Recently, Roussel-Gervais et al showed that overexpression of cyclin E in murine pituitary POMC cells leads to abnormal reentry into cell cycle of differentiated POMC cells and to centrosome instability. These alterations are consistent with the intermediate lobe hyperplasia and anterior lobe adenomas observed in these pituitaries [90]. As this chapter was in press, we published a germline transgenic zebrafish overexpressing PTTG targeting the pituitary POMC lineage, which recapitulated features pathognomonic of corticotroph adenomas including corticotroph expansion, partial glucocorticoid resistance, and pituitary cyclin E up-regulation, as well as metabolic disturbances mimicking hypercortisolism due to Cushing's disease [91]. Selective CDK inhibitors effectively targeted zebrafish and murine corticotroph tumor growth and hormone secretion [91]. A better understanding of the specific genetic and epigenetic alterations in human Cushing's disease will be necessary for selecting the appropriate combination of current treatments and/or developing new therapeutic approaches.

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