The Role of Genetic and Epigenetic Changes in Pituitary Tumorigenesis

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Abstract

Pituitary adenomas are one of the most common intracranial tumors. Despite their benign nature, dysregulation of hormone secretion causes systemic metabolic deterioration, resulting in high mortality and an impaired quality of life. Tumorigenic pathogenesis of pituitary adenomas is mainly investigated by performing genetic analyses of somatic mutations in the tumor or germline mutations in patients. Genetically modified mouse models, which develop pituitary adenomas, are also used. Genetic analysis in rare familial pituitary adenomas, including multiple endocrine neoplasia type 1 and type 4, Carney complex, familial isolated pituitary adenomas, and succinate dehydrogenases (SDHs)-mediated paraganglioma syndrome, revealed several causal germline mutations and sporadic somatic mutations in these genes. The analysis of genetically modified mouse models exhibiting pituitary adenomas has revealed the underlying mechanisms, where cell cycle regulatory molecules, tumor suppressors, and growth factor signaling are involved in pituitary tumorigenesis. Furthermore, accumulating evidence suggests that epigenetic changes, including deoxyribonucleic acid (DNA) methylation, histone modification, micro ribonucleic acids (RNAs), and long noncoding RNAs play a pivotal role. The elucidation of precise mechanisms of pituitary tumorigenesis can contribute to the development of novel targeted therapy for pituitary adenomas.

Key words: pituitary adenoma, tumorigenesis, cyclic adenosine monophosphate, cell cycle, growth factor

Introduction

The pituitary gland is the central mediator for peripheral endocrine homeostasis regulation by secretion of tropic hormones, such as adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), growth hormone (GH), prolactin, follicle-stimulating hormone (FSH), and luteinizing hormone (LH).¹ Pituitary adenomas are common, with 5–10% prevalence rates found at autopsy in postmortem studies,²,³ accounting for 15% of all intracranial tumors.⁴,⁵ Most common among these are prolactin (PRL)-producing pituitary adenomas (29%) or clinically nonfunctioning pituitary adenomas (NFPAs), derived from all cell types of the adenohypophysis, though mostly gonadotrophs, particularly FSH-producing adenomas. The prevalence of GH-producing pituitary adenomas is 15% and that of ACTH-producing pituitary adenomas is 10%, while TSH-producing pituitary adenomas are rare.⁶–⁹ An epidemiological study of 9,519 Japanese patients with pituitary adenoma revealed that 46% were diagnosed with NFPAs, 25% with PRL-producing adenomas, 22% with GH-producing adenomas, and 6% with ACTH-producing adenomas.¹⁰ Despite the mostly benign nature of the tumor, hormonal dysregulation and local expansion cause either an excess or impaired secretion of pituitary hormones, causing disturbance in growth, reproductive function, and metabolism, resulting in various morbidities, impaired quality of life, and increased mortality.⁴

Surgical resection is the first-line of treatment for pituitary adenomas, except for PRL-producing adenomas. Residual or recurrent tumors require re-operation, medical treatment, or radiation. An understanding of the physiological regulation and molecular mechanisms of pituitary hormone synthesis, secretion, and peripheral action has led to the development of targeted drugs such as dopamine agonists, somatostatin analogs, GH receptor antagonists, steroidogenic inhibitors, and glucocorticoid receptor antagonists.¹¹–¹⁷ In this regard, a better understanding of pituitary tumorigenesis is crucial for the development of novel targeted drugs.
for pituitary adenomas. In this review, we discuss human pituitary adenomas and animal models, as well as the involvement of genetic and epigenetic changes in pituitary tumorigenesis.

**Pathogenesis of Pituitary Adenomas**

Pituitary adenomas are considered to be of monoclonal origin, based on X-chromosome inactivation studies, suggesting that these tumor cells arise from a single cell. Therefore, it has been hypothesized that a mutation in the cell might cause pituitary adenomas as well as other tumors. Indeed, mutations in the GNAS gene have been reported as a cause of GH-producing pituitary adenomas. Furthermore, the analysis of pituitary adenomas related to hereditary syndromes has revealed several causal germline mutations in pituitary adenomas. For example, multiple endocrine neoplasia type 1 (MEN1), Carney complex (CNC), familial isolated pituitary adenomas (FiPAs), and succinate dehydrogenases (SDHs)-related paraganglioma syndrome, shows germline mutations in MEN1, PRKAR1A, CDKN1B, and SDHs genes, respectively, and loss of heterozygosity (LOH) at the affected locus in the tumor is generally observed (Table 1). However, the frequency of familial pituitary adenomas is less than 5% in patients with pituitary adenomas, demonstrating that the cause of most tumors remains unknown. On the other hand, somatic GNAS1 mutations were found in 30–40% of GH-producing pituitary adenomas, indicating that mutations contribute to the development of pituitary tumors (Fig. 1). Recently, epigenetic deregulation, including deoxyribonucleic acid (DNA) methylation, histone modification, nucleosomes remodeling, and ribonucleic acid (RNA) mediated targeting, have been shown to play a causative role in pituitary tumorigenesis. DNA methylation is a stable modification that leads to chromatin remodeling, resulting in transcriptional silencing without gene mutation. It occurs at cytosine residues in CpG islands, frequently located within the promoter region of the gene. This mechanism is regulated by DNA methyltransferases (DNMTs), namely DNMT1, DNMT3A, and DNMT3B. In contrast to DNA methylation, histone modifications are reversible, and can lead to either activation or repression of gene transcription, brought about by specific acetylation

**Table 1 Genetic changes in human pituitary adenomas and modified mice models with pituitary adenomas**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Human Mutations</th>
<th>Human LOH</th>
<th>Somatic Mutations</th>
<th>Mice Mutations</th>
<th>Mice LOH</th>
<th>Syndrome</th>
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<tbody>
<tr>
<td><strong>Genetic mutations</strong></td>
<td><strong>Somatic mutations</strong></td>
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<tr>
<td><strong>LOH</strong></td>
<td><strong>Mutations</strong></td>
<td><strong>LOH</strong></td>
<td><strong>Mutations</strong></td>
<td><strong>LOH</strong></td>
<td><strong>Mutations</strong></td>
<td><strong>LOH</strong></td>
</tr>
<tr>
<td>MEN1</td>
<td>11q13</td>
<td>+</td>
<td>+</td>
<td>+ (hetero)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>17q24.2</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>AIP</td>
<td>11q13.3</td>
<td>+</td>
<td>+</td>
<td>+ (hetero)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CDKN1B (p27kip1)</td>
<td>12p13</td>
<td>+</td>
<td>±</td>
<td>+ (homo)</td>
<td>NA</td>
<td>Downregulated</td>
</tr>
<tr>
<td>SDHs</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>NA</td>
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</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CDKN2C (P18ink4c)</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>PTTG1</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td>FGFR4</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>D2R</td>
<td>11q23</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>PRLR</td>
<td>5p13.2</td>
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or methylation lesions.\textsuperscript{33} Although several animal models of pituitary tumors have helped to identify potentially causative genes, few mutations of these genes have been detected in human pituitary adenomas. For example, retinoblastoma (Rb)-associated protein gene,\textsuperscript{34} pituitary tumor transforming gene (PTTG),\textsuperscript{35} high mobility group A (HMGA),\textsuperscript{36,37} and cyclin E (CCNE1)\textsuperscript{38} reportedly play an important role in pituitary tumorigenesis in mice; however, there have been no mutations in these genes in humans, suggesting a possibility of misregulation of expression levels or post-transcriptional regulation of these genes. DNMT3B is highly expressed in human pituitary adenomas, including GH-, PRL-, and ACTH-producing pituitary adenomas, as well as NFPA, compared to normal pituitary. Knockdown of DNMT3B in AtT20 mouse ACTH-producing pituitary adenoma cell line enhanced Rb expression.\textsuperscript{39} The promoter region of the Rb gene is frequently hypermethylated in pituitary adenomas.\textsuperscript{30,40} MicroRNAs (miRNAs) are endogenous small noncoding RNAs that bind to 3´-untranslated regions (3´-UTRs) of target mRNAs, and thus regulate gene expression.\textsuperscript{42} Deregulated miRNAs have been reported to regulate genes associated with pituitary tumorigenesis.\textsuperscript{1,43,44} These findings demonstrate a crucial role of epigenetic deregulation in pituitary tumorigenesis.\textsuperscript{26,45}

I. Genetic changes

Many genetic changes related to pituitary tumor development in humans and mice have been reported. These genes are summarized in Table 1.

1. Evidence in humans

Pituitary adenomas are mostly observed in sporadic conditions, but some also arise in familial tumor syndromes, and both show clonal expansion from a single cell. LOH in the tumor is generally observed in familial syndromes, and somatic mutation occurs in most sporadic tumors.

Germline mutations: MEN1, located on chromosome 11q13, encodes the protein menin.\textsuperscript{46} Heterozygous mutation in MEN1 is responsible for MEN1, an autosomal dominant syndrome, first identified in 1997.\textsuperscript{47} Germline mutation of the gene represents tumor development in the parathyroid glands, anterior pituitary, and endocrine pancreas.\textsuperscript{48} Nonsense or frameshift mutations lead to inactivation of the tumor suppressor function of menin.\textsuperscript{49} The penetrance of pituitary adenomas in patients with MEN1 varies from 15–50% in different series.\textsuperscript{50} Estimated prevalence of MEN1-associated pituitary adenomas is 2.7% in all pituitary adenomas.\textsuperscript{51} It has been reported that all cell types of anterior pituitary adenomas, except the true gonadotropinoma, have been reported in this group.\textsuperscript{52,53} Pituitary adenomas in patients with MEN1 represent larger size, more aggressive behavior, and reduced response to treatment as compared to non-MEN1.\textsuperscript{54} Plurihormonal expression is more frequently observed in MEN1-associated pituitary tumors.\textsuperscript{54,55} No specific histological difference in cellular and nuclear features or proliferative markers is observed between MEN1- and non-MEN1-associated pituitary tumors.\textsuperscript{55}

**Fig. 1** Enhanced cAMP signaling in pituitary adenomas. Activating somatic gain-of-function mutations in GNAS1 gene, which encodes α subunit of stimulatory G protein (Gαs), cause GH-producing pituitary adenoma. Loss of expression and/or function mutations in PRKAR1A gene results in Carney complex. PRKAR1A gene encodes type 1 regulatory subunit (R) of protein kinase A that inhibits the catalytic subunits (C) activated by an increase in intracellular cAMP levels. AC: Adenyl cyclase, CRE: cAMP response elements, cAMP: cyclic adenosine monophosphate, CREB: cAMP responsive element binding protein, CRHR: Corticotrophin releasing hormone receptor, D2R: dopamine receptor type 2, GH: growth hormone, GHRHR: growth hormone releasing hormone receptor, Gna: α subunit of inhibitory G protein, GnRHR: gonadotropin releasing hormone receptor, GPCR: G-protein coupled receptor, Gαs: α subunit of stimulatory G protein, p-CREB: phospho-CREB, PKA: protein kinase A, SSTR: somatostatin receptor.

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**Table 1** Genetic changes related to pituitary tumor development in humans

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Mutations</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNAS1</td>
<td>Encodes α subunit of stimulatory G protein</td>
<td>Loss of expression and/or function</td>
<td>GH-producing pituitary adenoma</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>Encodes type 1 regulatory subunit of protein</td>
<td>Mutations</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td>MEN1</td>
<td>Encodes menin</td>
<td>Germline mutation</td>
<td>Anterior pituitary tumor, parathyroid</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>glands, endocrine pancreas</td>
</tr>
<tr>
<td>HMGA</td>
<td>Encodes high mobility group A</td>
<td>Mutations</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td>CCNE1</td>
<td>Encodes cyclin E</td>
<td>Mutations</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td>Rb</td>
<td>Encodes retinoblastoma (Rb)-associated protein</td>
<td>Knockdown</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td>DNMT3B</td>
<td>Encodes DNA methyltransferase</td>
<td>Hypermethylation</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>Encodes type 1 regulatory subunit of protein</td>
<td>Mutations</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td></td>
<td>A</td>
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</table>
for ACTH, GH-producing pituitary adenomas are most common, while abnormal PRL secretion or PRL-producing pituitary adenomas were also involved in CNC. In somatomammotroph hyperplasia, which appears to predate adenomas, loss of heterozygosity (LOH) of PRKAR1A has not been observed consistently.

AIP, located on chromosome 11q13.3, encodes the aryl-hydrocarbon receptor interacting protein (AIP). Heterozygous inactivating mutations of AIP were observed in 15–20% of patients with FIPAs. LOH of AIP was identified in the pituitary adenoma. The penetrance of pituitary adenomas in patients with AIP mutations is 40–50% in families with GH-producing adenomas or PRL-producing adenomas. In addition, GH-producing pituitary adenomas associated with AIP mutations are generally large and resistant to somatostatin analogs.

CDKN1B gene, located on chromosome 12p13, encodes cyclin dependent kinase inhibitor p27kip1, which negatively regulates the Cdk2/Cyclin E and Cdk2/Cyclin A protein complexes and prevents cell cycle progression. Heterozygous loss of function CDKN1B mutations have been identified in patients with MEN4, an autosomal dominant disorder characterized by parathyroid and pituitary tumors. Approximately 3% of patients with clinical MEN1 without MEN1 mutations have a mutation in this gene. CDKN1B mutation was recently identified in AIP mutation-negative patients with FIPA.

SDHx genes encode the subunits of SDH or mitochondrial complex II. A mutation in this gene is related to familial paraganglioma syndrome and several tumors including pituitary adenomas. LOH of SDHD has been reported in pituitary tumors, though the incidence of SDH mutation in pituitary adenomas may be rare.

Somatic mutations: GNAS1, located on chromosome 20q13, encodes G protein α-subunit (G\(_{\alpha}\)), which couples numerous hormonal signaling to adenylyl cyclase. Ligands that bind G\(_{\alpha}\)-coupled receptors stimulate intracellular cyclic adenosine monophosphate (cAMP) production (Fig. 1). Activating mutations of the gene are identified as missense mutations, which lead to amino acid substitutions of either residue Arg201 or Gln227, resulting in decreased intrinsic GTPase activity and increased cAMP. Somatic mutations of GNAS are identified in 30–40% of GH-producing pituitary adenomas. In Japan, it has been reported that 53% of GH-producing adenomas exhibited somatic GNAS mutations.

PIK3CA, located on chromosome 3p26.3 encodes the catalytic subunit PIK3CA of class IA PI3-Kinase, which exists as a heterodimer of p110 catalytic- and p85 regulatory-subunits, upstream of the AKT signaling pathway. Activating somatic mutations in PIK3CA at exon 9 and 20 have been identified in pituitary adenomas, including ACTH-producing, PRL-producing, plurihormonal, and NFPAs. Interestingly, this mutation was seen in 8.8% of invasive

![Fig. 2 The aberrant regulation of cell cycle in pituitary tumorigenesis. Targeted deletion in Rb gene results in pituitary adenomas depending of a transcription factor E2F, which induces G1 to S phase entry of cell cycle in mice. Cyclin dependent kinases (Cdks), cyclin D and E phosphorylate and inactivate Rb protein. Cyclin D and E are inactivated by Cdk inhibitors (Cdkis) p16, p18, and p27. Skp2 negatively regulates p27 by protein degradation. An activation of growth factor including EGF receptor suppresses Cdks. An overexpression of architectural transcriptional factors HMGAs induces pituitary adenomas in E2F-dependent manner. MEN1 gene encodes menin. This is a tumor suppressor gene, which mediated by Cdk4. Pituitary tumor transforming gene (PTTG) regulates metaphase-anaphase transition as a securin. An overexpression of PTTG induces pituitary adenomas in a downstream of Rb/E2F. E2F1: E2F transcription factor 1, HMGA: high mobility group A.](Image 298x570 to 535x718)
pituitary tumors, while no mutations were detected in noninvasive tumors.88)

2. Animal models

Consistent with human genetic mutation analyses, several mouse models that develop pituitary adenomas and hyperplasia have been generated. Although these models show many phenotypes similar to human pituitary adenomas, several notable differences have also been observed.

Men1+ mice develop tumors in the endocrine pancreas and parathyroid within 9 months of age and pituitary tumors within 12 months.89,90) The tumors developed in Men1+ mice show LOH and Cdk4 is a critical downstream of Men1-dependent tumor suppression, while Cdk2 is dispensable91) (Fig. 2). Menin, encoded by Men1, interacts with double-stranded DNA and plays a crucial role in regulating cell proliferation by blocking the cell cycle.92,93) Menin reportedly attenuates the effect of activin on PRL and GH suppression in a Pit-1-dependent manner.92,93) Menin reportedly attenuates the effect of activin on PRL and GH suppression in a Pit-1-dependent manner.92,93)

Aip+/− mice are tumor-prone and tend to develop tumors in cAMP-responsive tissues and sarcomas.96,97) However, these mice do not show any pituitary tumors. Pituitary-specific knockout of PRKAR1A (Prkar1a-pitKO) mice, generated using the rat GHRH receptor promoter to drive Cre expression and crossing them to PRKAR1Alop mice, developed pituitary tumors that were multiple and positive for GH-, PRL-, TSH-, and Pit-1-specific strains. Serum GH levels revealed a 3-fold elevation as compared to controls.98) The PKA catalytic subunit has been shown to be downstream of the PKA pathway,99) and its constitutive active mutation in adrenocortical cells results in unilateral cortisol-producing adrenal adenomas, suggesting a common pathway in the tumorigenesis50) (Fig. 1).

Aip+ mice are phenotypically normal and fertile.100) The hypomorphic Aip mouse model, expressing 10% normal Aip, shows a patent ductus venosus. Similar phenotypes have been shown in hypomorphic aryl hydrocarbon receptor nuclear translocator (ARNT) mice,101) a well-known interactive partner of AIP,102) indicating the important role of AIP is mediated by its interaction with ARNT.103) No pituitary tumors develop in these animals. In contrast, heterozygous Aip mutations generated by insertion of a gene trap vector construct into an intronic region of genomic DNA between Aip exons 2 and 3 showed 100% of pituitary adenomas, particularly GH-secreting tumors.104) This difference may be due to different sub-strains used for inbreeding or the difference in the placement of the germline mutation to induce the inactivation of Aip.104) Ah receptor nuclear translocator 1 and 2 (ARNT1 and ARNT2) have been shown as possible mediators of AIP function.104) In addition, AIP protein interacts with several proteins including AhR, heat shock protein 90, cAMP, phosphodiesterases (PDE4A5 and PDE2A), epidermal growth factor receptor (EGFR), ret proto-oncogene (RET), and peroxisome proliferator-activated receptor gamma (PPARγ).105,106)92,93) suggesting a possible role in angiogenesis and cell proliferation.

p27−/− mice, exhibiting increased body weight and multi-organ hyperplasia, develop intermediate lobe pituitary tumors expressing ACTH.105,106) Deletion of p27 in the pituitary by POMC-Cre generates intermediate lobe pituitary tumors.107) MENX-affected rats, which display diminished expression of p27 due to a Cdkn1b mutation, develop multiple endocrine tumors including pituitary tumors.108) Recently, the deletion of S-phase kinase-associated protein 2 (Skp2),109 the third ubiquitin ligase for p27, has been shown to block co-deletion of Rb and Trp53 and induced pituitary tumorigenesis probably mediated by p27 accumulation in the nucleus.110) This suggests that p27 plays a key role as a tumor suppressor in pituitary tumorigenesis (Fig. 2).

Rb+ mice are not predisposed to retinoblastoma, but to high frequency of pituitary adenomas in an E2F-dependent manner.110,111) Rb is a key cell cycle regulator and tumor suppressor, which serves to inhibit the transcription of multiple genes required for entry into the S-phase. Inactivation of Rb function by several Cdns including cyclin D1/Cdk4 induces tumorigenesis.112) Pituitary-specific deletion of Rb by using POMC-Cre results in development of intermediate lobe pituitary tumors, which are completely prevented by Skp2 deletion, demonstrating the essential role of Skp2 in the downstream of Rb pathway113) (Fig. 2).

Two families of Cdk inhibitors exist, namely the INK4a/ARF (p15, p16, p18) and Cip/Kip families (Fig. 2). The INK4a/ARF family inhibits cyclin D1/Cdk4, whereas the Cip/Kip family inhibits cyclin E/Cdk2. Both families of Cdk inhibitors normally act as tumor suppressors by preventing entry into the S phase in an Rb-mediated manner.1,113,115) Genetic deletion of Cdk inhibitors in mice has generated pituitary tumor animal models, demonstrating a pathogenic significance for cell cycle abnormality in pituitary tumorigenesis, at least in animal models. p16INK4A-deficient mice develop GH-producing pituitary adenomas with increasing body size and organomegaly. p27kip1-deficient mice demonstrate similar phenotypes,4,105,116) p18INK4C/p27kip1 double-null mice exhibit more aggressive pituitary tumors than single knockout mice and...
PTTG encodes pituitary tumor transforming gene 1 (PTTG1), the mammalian protein securin, which is a transcriptional activator\(^{119,120}\) (Fig. 2). PTTG1-null mice exhibit pituitary hypoplasia and when crossbred with Rb\(^{+}\) mice, which develop high-penetrance pituitary tumors, showed significant delay in developing pituitary tumors.\(^{121}\) This suggests that PTTG1 is downstream of Rb/E2F in pituitary tumorigenesis.\(^{122}\) Pituitary-specific PTTG1 overexpression in mice by using \(\alpha\)-subunit of glycoprotein hormone (oGsu) promoter generates focal pituitary hyperplasia and pituitary tumors, inducing aneuploidy and chromosomal instability.\(^{35,123}\) POMC-Pttg overexpression in zebrafish generates ACTH-producing pituitary tumors and treatment with the Cyclin E inhibitor Roscovitine attenuates tumor growth and hormone secretion.\(^{118}\) (Fig. 2).

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HMGA encode HMGA proteins that are known as architectural transcriptional factors, namely HMGA1a, HMGA1b, and HMGA1c from HMGA1 gene, and MHGA2 from HMGA2 gene.\(^{37}\) Both HMGA1 and HMGA2 transgenic mice develop mixed GH/PRL-secreting pituitary adenomas.\(^{36,37}\) Absence of E2F1 suppresses these pituitary tumors in HMGA2 transgenic mice, suggesting that the cell cycle is deregulated by HMGA2 in pituitary tumorigenesis.\(^{37}\) (Fig. 2).

Overexpression of transforming growth factor (TGF)-\(\alpha\), an EGFR ligand, using PRL promoter in mice, generates PRL-producing pituitary adenomas,\(^{124}\) suggesting the involvement of EGFR in pituitary tumorigenesis. Additionally, inhibition of EGFR and its family of ErbB kinases has been shown to suppress hormone secretion and cell proliferation\(^{125-127}\) (Fig. 2). Fibroblastoma growth factor receptor 4 (FGFR4) is a member of the FGFR family, which includes FGFR1 through 4. FGFR4 overexpression is associated with chemotherapy resistance and single nucleotide polymorphisms in the gene locus have been identified in breast cancer.\(^{128}\) FGFR4 kinase-containing, N-terminal truncated variant of FGFR4 has been identified as pituitary tumor-derived (ptd)-FGFR4.\(^{129}\) Overexpression of ptd-FGFR4 in mice generates PRL-producing pituitary adenomas.\(^{130}\) Dopa

Dopa
tumors due to hypermethylation of its promoter.\textsuperscript{141} \n
*GNAS1* is an imprinting gene that is regulated by DNA methylation. *GNAS1* activating mutation in GH-producing pituitary adenomas or MAS is present on the active maternal allele. This is different from normal pituitary, in that Gs\textsuperscript{α} expression has also been observed in the non-mutated paternal allele, demonstrating the impact of *GNAS* imprinting relaxation on pituitary tumorigenesis.\textsuperscript{142} \n
**Histone modification**: Tail acetylation or methylation of histone lysine residues can lead to either activation or repression of gene transcription. Many of the histone modifications are misregulated in cancer.\textsuperscript{133} DNA methyltransferase 3B (DNMT3b), which encodes a protein that produces 5-methylcytosine by adding a methyl group to a cytosine base, was shown to be overexpressed in pituitary adenomas. Down-regulation of DNMT3b in AtT20 mouse corticotroph adenoma cells results in histone 3 acetylation and diminished histone methylation in Rb, p21, and p27.\textsuperscript{39} *IK6* is a dominant-negative isoform of the transcription factor Ikaros, a family of zinc-finger DNA-binding proteins. *IK6* has been identified in pituitary adenomas and has been shown to be epigenetically regulated through histone and DNA modifications.\textsuperscript{143,144} \n
**miRNAs**: miRNAs are small, single-stranded, non-coding RNA molecules, which consist of approximately 22 nucleotides. miRNAs bind to sequences at 3′ untranslated regions of mRNAs, resulting in post-transcriptional silencing.\textsuperscript{145} It has been reported that miRNA dysregulation plays a crucial role in the progression of cancer.\textsuperscript{146} The analysis of expression profiles and functional properties in pituitary adenomas has revealed that miRNAs play a significant role in pituitary tumorigenesis\textsuperscript{24,44} (Table 2). \n
*AIr* was identified as a target for miR-107, which is overexpressed in pituitary adenomas.\textsuperscript{147} BM1 polycomb ring finger oncogene 1 is a target for miR-128, which is downregulated in GH-producing pituitary adenomas leading to phosphatase and tensin homolog (PTEN) suppression.\textsuperscript{148} *E2F1* is the target for miR-326 and miR-603, while *HMGA1/HMGA2* is the target for miR-15, miR-16, miR-26a, miR-34b, miR-548-3p, miR-196a2, and let-7a, which are downregulated in pituitary adenomas.\textsuperscript{149,150} miR-326, miR-432, and miR-570 are also downregulated in pituitary adenomas that target *HMGA2*.\textsuperscript{149} *PRKCD*, a serine/threonine kinase involved in proliferation, apoptosis and cell cycle regulation, is a direct target for miR-26a, which is overexpressed in ACTH-producing pituitary adenomas.\textsuperscript{151} *PTEN*, a suppressor of the PI3K/AKT signaling pathway, is identified as a direct target for miR-26b, which is overexpressed in GH-secreting pituitary adenomas.\textsuperscript{148} Arginyl-tRNA synthetase (RARS), a part of the aminoacyl-tRNA synthetase complex, is a target for miR-16-1, whose expression levels are low in pituitary adenomas.\textsuperscript{152} *SMAD3* is a target for miR-135a, miR-140-5p, miR-582-3p, miR-582-5p, and miR-938, which are overexpressed in NFPAs, as compared to normal pituitaries.\textsuperscript{153} Vascular endothelial growth factor receptor 1 (*VEGF-R1*) is a target for miR-24-1, which is downregulated in

<table>
<thead>
<tr>
<th>Target genes of miRNAs</th>
<th>Upregulated miRNAs</th>
<th>Downregulated miRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>AIP</em></td>
<td>miR-107</td>
<td>miR-128</td>
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<tr>
<td><em>BMI1</em></td>
<td></td>
<td>miR-326, miR-603</td>
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<tr>
<td><em>E2F1</em></td>
<td></td>
<td>miR-15, miR-16, miR-26a, miR-34b, miR-548c-3p, miR-196a2, let-7a</td>
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<tr>
<td><em>HMGA1</em> and <em>HMGA2</em></td>
<td>miR-26a</td>
<td>miR-326, miR-432, miR-570</td>
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<tr>
<td><em>HMGA2</em></td>
<td>miR-26b</td>
<td>miR-16-1</td>
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<tr>
<td><em>PRKCD</em></td>
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<td>miR-24-1</td>
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<tr>
<td><em>PTEN</em></td>
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<tr>
<td><em>RARS</em></td>
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<tr>
<td><em>SMAD3</em></td>
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<td>miR-16-1</td>
</tr>
<tr>
<td><em>VEGF-R1</em></td>
<td>miR-128a, miR-155, miR-516-3p</td>
<td></td>
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<tr>
<td><em>Wee1</em></td>
<td>miR-26a</td>
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<td><em>ZAC1</em></td>
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pituitary adenomas. Wee1, an inhibitor for Cdk1, is identified as a target for miR-128a, miR-155, and miR-516-3p, which are overexpressed in pituitary adenomas. ZAC1, also called as PLAG1, which is a downstream component of a particular signal pathway involving AIP, is a target for miR-26a, which is overexpressed in pituitary adenomas.154)

**Lnc RNAs:** LncRNAs are non-protein coding transcripts, longer than 200 nucleotides. LncRNA are involved in the regulation of molecules related to the cell cycle, including CDK inhibitors, CDKs, Rb, and p53, in addition to functioning as epigenetic regulators, transcription factor regulators, post-transcription regulators, and protein scaffolds.156)

Maternally expressed gene 3 (MEG3), located on chromosome 14q32, belongs to the DLK1-MEG3 imprinting locus, containing multiple maternally and paternally imprinted genes. MEG3 encodes IncRNA and is downregulated in pituitary adenomas, especially in NFPAs. MEG3 stimulates p53-dependent transcription and acts as a tumor suppressor gene.160)

**Future Directions and Conclusion**

Accumulating evidence suggests that not only genetic changes, but also epigenetic changes play an essential role in the development of pituitary adenomas. Both clinical data and the analysis of animal models are important; however, there are substantial differences between species. In this regard, it is important to establish a human tumor experimental model.

To develop novel therapeutic targeted drugs, it is essential to identify the pathway responsible for pituitary tumorigenesis. Somatostatin analogs are important targeted drugs, that inhibit the pathways essential for GH secretion in GH-producing pituitary adenoma. Recent findings suggest that ErbB receptors or Skp2, which is an upstream effector of CDK inhibitors, could be useful as a novel strategy for targeted therapy.114,126,161)

In conclusion, human genetic analysis and establishment of animal models have revealed the mechanisms of pituitary tumorigenesis. Further clarification of underlying mechanisms can contribute to the development of novel targeted drugs for pituitary adenomas.

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**Conflicts of Interest Disclosure**

There is no COI to be disclosed.

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