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Recent Progress in Studies of Pituitary Tumor Pathogenesis

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The mechanisms of tumorigenesis of the human pituitary have been elucidated to a limited extent. Classically, pituitary tumor formation was shown to be induced by thyroidectomy and estrogen administration. Molecular biological and immunohistochemical studies have revealed several aspects of pituitary tumorigenesis. Trans-lineage cell differentiation has been shown to be induced by the aberrant expression of transcription factors and co-factors, such as Pit-1, Prop-1, and estrogen receptor. Defects or overexpression of cell cycle regulators, such as CDK inhibitors, PTTG, and GADD45γ, result in the abnormal proliferation of pituitary cells. Recently, epigenetic regulation has been suggested to be related to pituitary tumor formation. This article presents a review and update of recent progress in studies of the development and differentiation of pituitary tumors.

Key Words: Pituitary tumor; transcription factor; trans-lineage differentiation; cell cycle regulator; epigenetics.

Introduction

The pituitary anterior lobe has been shown to secrete a total of six hormones—growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). TSH, FSH, and LH are glycoprotein hormones, all of which consist of a common alpha-subunit (αSU) and a specific beta-subunit (βSU). The pituitary anterior lobe is composed of hormone-secreting cells and folliculostellate cells. In general, GH, PRL, ACTH, and TSH are secreted from different cells, while FSH and LH are secreted from the same cells. FS cells are positive for S-100 protein but lack hormone secretion. Immunohistochemistry is an important technique to clarify the functional differentiation of pituitary cells.

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These hormone-secreting cells develop from common progenitor cells and can be divided into three cell lineages —ACTH-lineage, FSH/LH-lineage, and GH-PRL-TSH-lineage (Pit-1 lineage)—according to the process of their functional differentiation (Fig. 1) *(27)*. Recent molecular studies have indicated that particular transcription factors and their combinations with several co-factors regulate the specification of hormone-secreting cells during the development of the pituitary gland *(36)*.

Human pituitary adenomas are clinically subdivided into functioning and non-functioning adenomas. The former group includes GH-producing adenomas (GHoma), PRL-producing adenomas (PRLoma), TSH-producing adenomas (TSHoma), ACTH (POMC)-producing adenomas (ACTH-oma), and FSH-producing adenomas (FSHoma). The non-functioning adenomas (NFoma) are frequently positive for gonadotropin subunits, such as αSU, FSH βSU, and LH βSU.

This article presents a review and update regarding the mechanisms of development and differentiation of pituitary adenomas.

Pituitary Stimulation by Hormonal Signals

PRL-producing cell hyperplasia or adenomas were induced by continuous estrogen administration in rodents *(20, 28)*. The overexpression of LH in transgenic (Tg) mice resulted in the formation of ovarian cysts and granulosa cell tumors, and then PRLoma, Ghoma, and TSHoma in the pituitary *(32)*. However, estrogen replacement following ovariectomy induced only the expansion of lactotrophs in the LH Tg mice *(25)*. These results indicated that estrogen is sufficient for the development of PRLoma, whereas GHoma and TSHoma formation are dependent on other signals from ovary. Thyroidectomy is known to induce pituitary tumor formation *(10)*. Therefore, inappropriate feedback signals are important factors involved in pituitary tumorigenesis.

Some hypothalamic factors, which stimulate the production and secretion of pituitary hormone, also induce adenoma formation. The chronic administration of corticotrophin-releasing factor was shown to markedly increase the number of ACTH-producing cells in the anterior pituitary of rats *(11)*. In growth hormone-releasing hormone (GHRH) Tg mice, somatotrophs and mammosomatotrophs developed in the pituitary by 8 mo of age *(2,22)*. Osamura et al.

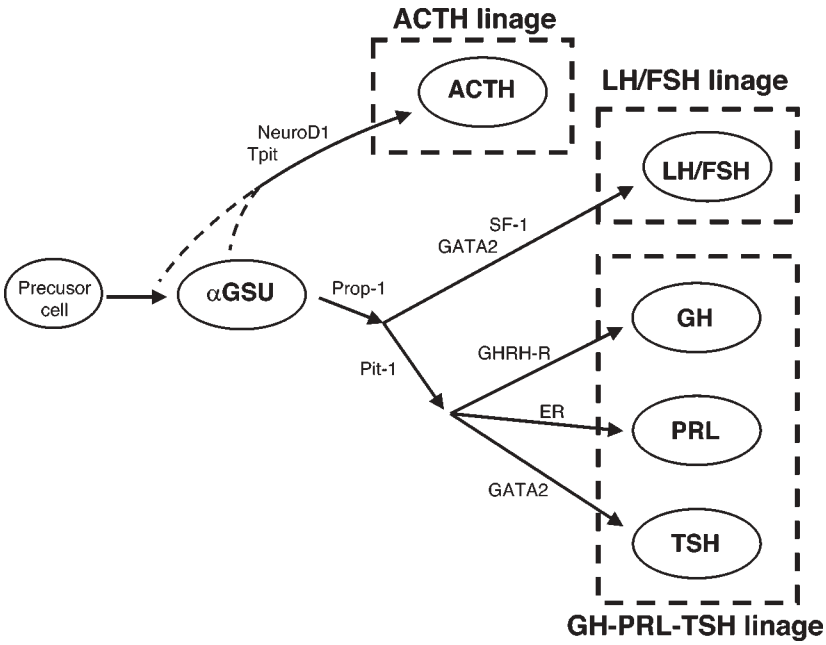

Fig. 1. Three cell lineages in pituitary development. Pituitary hormone–producing cells are differentiated from common precursor cells and can be divided into three cell lineages, i.e., ACTH-lineage, FSH/LH-lineage, and GH-PRL-TSH-lineage, according to the developmental process regulated by particular combinations of transcription factors and co-factors.

(29) discussed the GHRH autocrine–paracrine mechanisms involved in the induction of pituitary adenoma formation.

Transcription Factors in Pituitary Adenomas

The cloning of the pituitary-specific transcription factor, Pit-1 *(4,13)*, stimulated the application of molecular biological approaches to analysis of the mechanisms of development and differentiation of the pituitary gland and its tumors. Pit-1 is known to regulate the functional differentiation of GH-PRL-TSH cell lineage. GHRH receptor *(21, 35)*, estrogen receptor *(34)*, and GATA-2 *(38)* are co-factors involved in specification of pituitary cells into GH-, PRL-, and TSH-producing cells, respectively. Molecular biological and immunohistochemical studies have indicated that functioning pituitary adenomas exhibit a combination of transcription factors and co-factors similar to those seen under physiological or developmental conditions *(33)*. These results suggest that the functional development of functioning pituitary adenomas is regulated by molecular mechanisms similar to those under physiological conditions.

The expression of Pit-1 is required for the production of GH, PRL, and TSH in pituitary cells and pituitary adenoma cells. Miyai et al. *(24)* applied the siRNA technique to suppress Pit-1 expression in GH-producing MtT/S cells. The immunoreactivity for Pit-1 decreased in the fraction of siRNA-transfected cells, while no changes were observed in expression of actin or tubulin used as internal controls following transfection. Moreover, double staining for Pit-1 and GH indicated that GH production was reduced only in MtT/S cells in which Pit-1 immunoreactivity was decreased by siRNA transfection (Fig. 2A,B). Interestingly, Lee et al. *(19)* reported that tail vein injection of an adenovirus vector carrying the rat Pit-1 gene activated GH, PRL, and TSH-β gene expression in the mouse liver. These results strongly indicated the presence of Pit-1-dependent mechanisms of hormone production.

Trans-lineage Differentiation of Pituitary Adenomas

Multi-hormone-producing adenomas are frequently observed in human pituitary adenomas. Occasionally, functioning adenomas produce hormones that belong to a different lineage, such as ACTHoma with GH production *(37)* and FSHoma with GH or ACTH production *(30)*. In ACTHoma with GH production, immunohistochemical analyses indicated the aberrant expression of Pit-1 in addition to NeuroD1, although ACTH-producing cells usually express NeuroD1 and Tpit, but not Pit-1 (Figs. 2C,D,E) *(37)*.

Kurotani et al. *(18)* demonstrated the induction of new GH expression in the mouse pituitary ACTHoma-derived cell line, AtT-20, by transfection with the Pit-1 gene. These cells were considered a good experimental model for analysis of aberrant expression of transcription factors and trans-lineage differentiation of hormone-producing tumor cells.

Cell Cycle Regulators in Pituitary Adenomas

Some cell cycle regulators have been shown to be related to pituitary tumorigenesis.

The CDK inhibitors, which include two families, INK4 and CIP1/KIP1, have been shown to be responsible for several growth-inhibition signals, such as cell-to-cell contact inhibition and DNA damage. Therefore, deficiencies in some

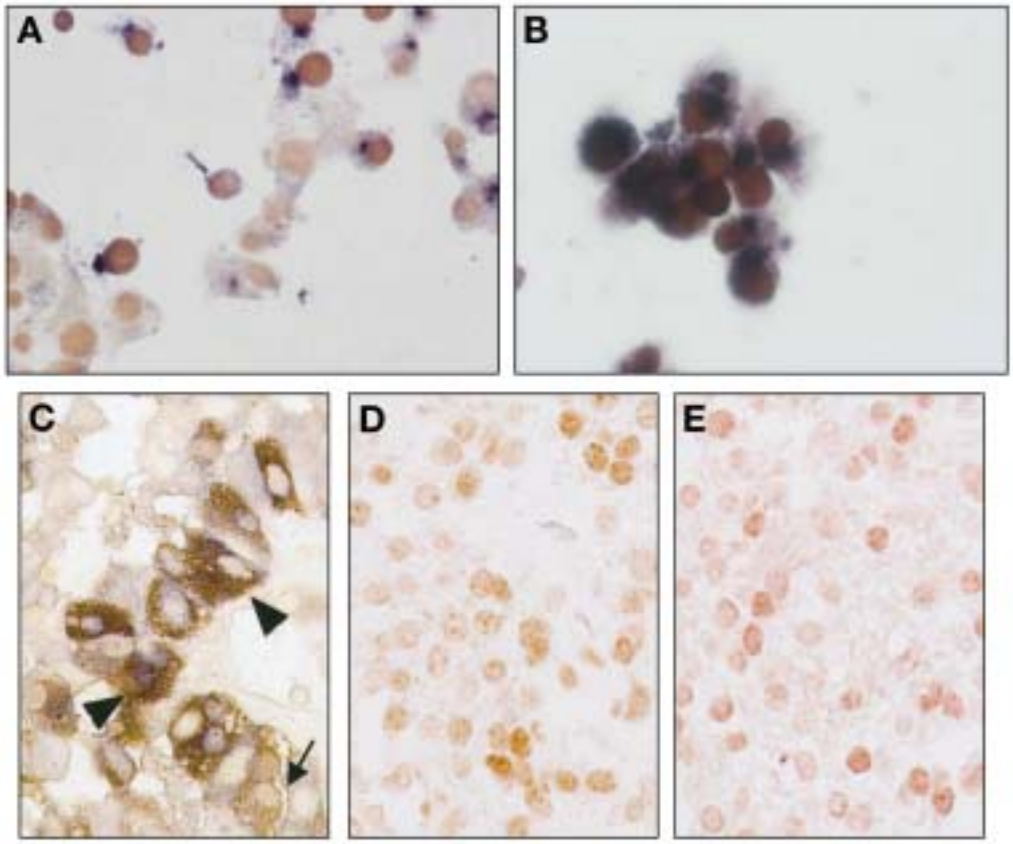

Fig. 2. Function of Pit-1 in pituitary adenomas. (**A,B**) Immunocytochemical analysis of Pit-1 knockdown MtT/S cells by siRNA transfection. Silencing of the Pit-1 gene by siRNA induced a marked decrease in GH production (**A**) as compared with normal MtT/S cells (**B**). Immunoreactivities of Pit-1 and GH are indicated in brown and blue, respectively. (**C–E**) Histochemical analysis of human ACTH-producing adenomas with GH production. GH (blue) and ACTH (brown) were produced simultaneously in the same adenoma cells (C, arrowhead), while some adenoma cells produced only ACTH (C, arrow). The detection of NeuroD1 (D) and Pit-1 (E) indicated aberrant expression of transcription factors.

CDK inhibitors induce tumor formation. Immunohistochemical analysis showed that the expression level of p27, which is a member of the CIP1/KIP1 family, was markedly reduced in human ACTHoma as compared with other types of pituitary adenomas *(16)*. In addition, the disruption of p27 in mice resulted in tumors of the pituitary intermediate lobe and multiorgan hyperplasia *(15,26)*. Loss of p18, which belongs to the INK4 family, leads to a gradual progression from intermediate lobe pituitary hyperplasia in young mice to adenoma with nearly complete penetration by 10 mo of age *(9)*. Moreover, p18 and p27 double KO mice invariably died from pituitary adenomas by 3 mo *(9)*. These results clearly indicated the presence of tumorigenic mechanisms regulated by CDK inhibitors.

Recently, pituitary tumor transforming gene (PTTG) and GADD45γ, a member of the growth arrest and DNA damage-inducible gene family, were isolated as new regulators of pituitary tumorigenesis *(31,42)*.

PTTG, which is also known as securin, has been identified from a rat pituitary tumor-derived cell line, GH4 *(31)*. While PTTG inhibits the progression of the cell cycle during mitosis *(43)*, PTTG promotes the expression of the growth factors, FGF-2 *(5)* and VEGF *(23)*, and also the apoptotic factors, p53 and c-myc *(12)*. In our immunohistochemical study, PTTG was shown to be overexpressed in pituitary adenomas. In particular, PTTG expression level was higher in GH-producing adenomas similar to the results reported by Zang et al. *(40)*. Abbud et al. *(1)* generated PTTG-transgenic mice that developed LH-, TSH-, and GH-producing cell hyperplasia and adenoma in the pituitary. Therefore, the overexpression of PTTG is considered a candidate involved in initiation or promotion of pituitary tumor formation.

On the other hand, GADD45γ, also known as cytokine response 6 (CR6), causes cell growth arrest and promotes apoptosis *(41)*. Although GADD45γ mRNA was found in all normal pituitary tissues, the expression of GADD45γ was not detected in the majority of GHoma, PRLoma, and NFoma. These observations indicate that GADD45γ is a growth suppressor in pituitary cells and the loss of its expression is an important characteristic of pituitary adenomas.

Epigenetics in Pituitary Adenomas

Gene expression is not determined solely by the DNA sequence, and is also dependent on epigenetic phenomena,

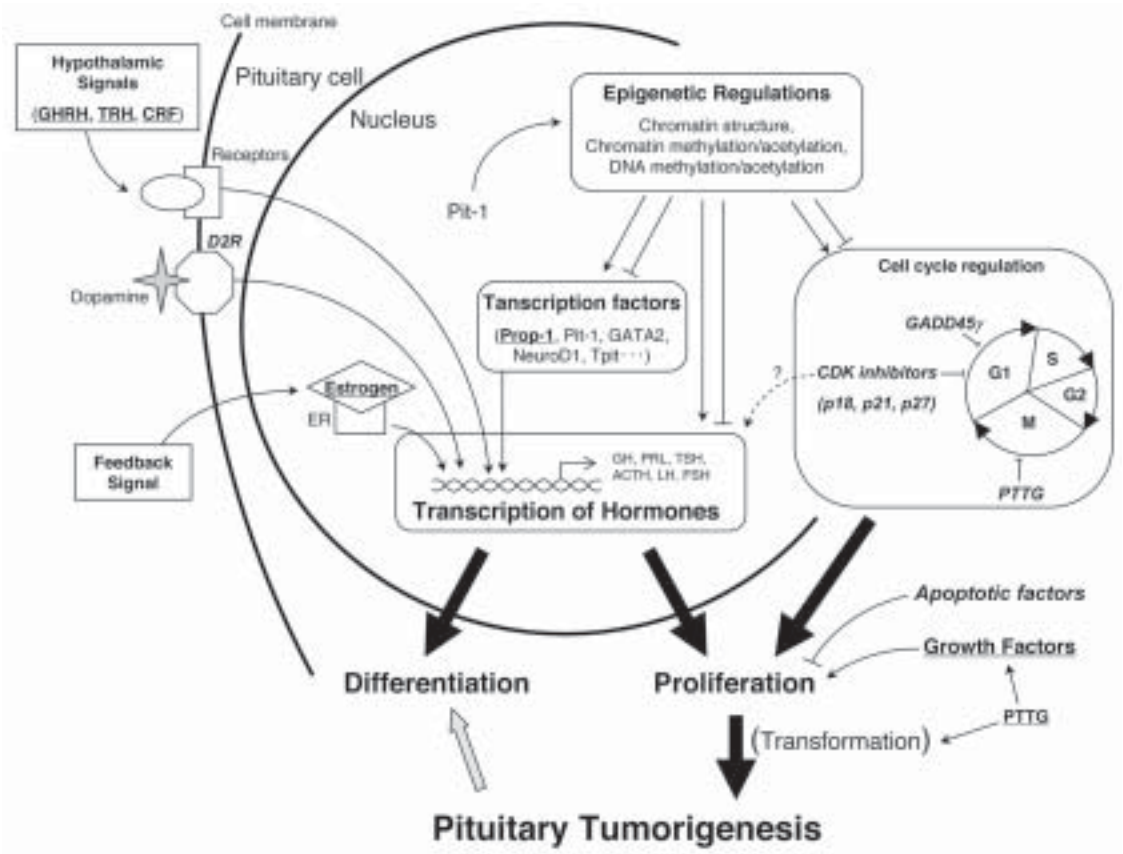

Fig. 3. Several factors affect pituitary tumorigenesis. Underline, Pituitary tumorigenesis-promoting factors; *Italic*, suppressing factors.

defined as gene-regulating activities that do not involve changes to the base sequence and that can persist through one or more generations *(39)*. The mechanism for establishment of epigenetic properties involves post-translational modifications of histones, the arrangement of nucleosomes, and higher-order structures of DNA–chromatin complexes, such as loops, as well as methylation of cytosine in DNA *(8)*.

Pituitary hormone expression is also regulated by epigenetic mechanisms. The coding region of the PRL gene was shown to be demethylated, while that of the GH gene was higher methylated in the pituitary glands of pregnant and lactating rats in which the expression level of PRL was elevated and GH expression was inhibited *(17)*. These results indicated that hormone expression in the pituitary gland is regulated by site-specific DNA methylation.

Recently, new roles of some transcription factors in epigenetic regulation of hormone expression in the pituitary gland were reported. The pituitary-specific transcription factor, Pit-1, can change the chromatin structure of the PRL promoter region *(14)*. Ikaros, a zinc finger transcription factor, selectively deacetylates the histone 3 residue on the GH promoter and acetylates histone 3 on the PRL promoter *(7)*. These chromatin structures affect the binding of activators or inhibitors of gene expression. The aberrant expression of transcription factors related to pituitary tumorigenesis, as described above, suggests strongly that epigenetic chromatin structures are involved in regulation of the development and/or differentiation of pituitary adenomas.

Epigenetic mechanisms are also expected to be involved in the regulation of hormone expression in pituitary tumors. Durrin et al. *(6)* found two DNase I-hypersensitive sites located 5' of the PRL gene in pituitary and pituitary tumors but none were found in the livers of estrogen-treated Fischer 344 rats. As the sensitivity to DNase I digestion is an indicator of the methylation state of DNA, these results suggested that the transcription of the PRL gene was activated in the normal pituitary and in pituitary adenoma, and inhibited in the liver.

Bahar et al. *(3)* reported that the loss of GADD45γ expression, which was detected in the majority of pituitary adenomas, was associated with methylation of 23 CpG islands within this gene. The methylation-associated gene silencing of the GADD45γ was also found in the pituitary tumor cell line, AtT-20. They also found re-expression of GADD45γ in AtT-20 cells following treatment with demethylating agents. These findings indicated the possible mechanisms of pituitary tumorigenesis associated with epigenetic changes of particular genes.

A number of factors and conditions have been shown to be related to pituitary tumorigenesis (Fig. 3). However, the

precise mechanisms of development and differentiation of pituitary tumors have not been clarified. Therefore, the existence of complicated pathways has been postulated.

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