Pituitary

Mohamad Maghnie^a, Andrea Secco^a and Sandro Loche^b

^aDepartment of Pediatrics, Istituto di Ricovero e Cura a Carattere Scientifico, Giannina Gaslini, University of Genoa, Italy

^bOspedale Regionale per le Microcitemie, Cagliari, Italy

Mechanism of the year Genes and pituitary adenomas

Rab18 is reduced in pituitary tumors causing acromegaly and its overexpression reverts growth hormone hypersecretion

Vazquez-Martinez R, Martinez-Fuentes AJ, Pulido MR, Jimenez-Reina L, Quintero A, Leal-Cerro A, Soto A, Webb SM, Sucunza N, Bartumeus F, Benito-Lopez P, Galvez-Moreno MA, Castano JP, Malagon MM
Department of Cell Biology, Physiology, and Immunology, Campus de Rabanales, Edificio Severo Ochoa, University of Cordoba, Cordoba, Spain

J Clin Endocrinol Metab 2008;93:2269–2276

Background: Rab proteins are GTPases involved in the coordination of intracellular membrane transport. Anomalies in these mechanisms cause diseases due to dysregulation of secretory activities. The aim of the present study was to evaluate the role of Rab18 in somatotrope tumorigenesis leading to acromegaly.

Methods: Analysis of pituitary samples was undertaken from 9 patients with acromegaly and 9 patients with non-functioning pituitary adenoma (NFPA) who had undergone endoscopic trans-sphenoidal surgery. Cell dispersion and culture, PCR amplification, immunoelectron microscopy, immunofluorescence microscopy and immunostaining studies, as well as Rab18 transfection experiments, were performed.

Results: Pituitary adenomas from patients with acromegaly exhibited both reduced Rab18 mRNA levels and protein content compared to NFPA. By means of immunoelectron microscopy, lower levels of Rab18 on the surface of GH-containing secretory vesicles were documented in acromegalic subjects compared to NFPA. The secretory activity of individual somatotropes from pituitary samples of acromegalic patients was enhanced compared to that of somatotropes from NFPA. The overexpression of Rab18 reduced by 40% the secretory activity of somatotropinoma cells in response to GHRH stimulation.

Conclusion(s): This study provides evidence that molecular anomalies of the intracellular molecule transport system can affect GH secretory levels in pituitary tumor cells of patients with acromegaly. The ascertainment of the role of Rab18 in the modulation of GH secretion in somatotropinoma cells opens the way for new diagnostic and therapeutic approaches to acromegaly.

Rab proteins, members of the Ras-related small GTPase superfamily of proteins, comprise a complex family of small GTPases involved in the regulation of intracellular membrane trafficking and reorganization. A recent genome analysis revealed that the Rab family is composed of more than 70 members in humans. The specific functions of most of GTPases remain to be elucidated.

The facilitating role of these GTPases in vesicle transport has been demonstrated in pituitary cells, adrenal chromaffin cells, and pancreatic β -cells. Recently, Rab18 was identified as a new inhibitory player in the secretory pathway in neuroendocrine cells. In adrenal chromaffin PC12 cells and pituitary AtT20 cells, Rab18 is located at the cytosol, but associates with a subpopulation of secretory granules after stimulation of the regulated secretory pathway, strongly suggesting that induction of secretion provokes Rab18 activation and recruitment to these organelles. These findings suggest that Rab18 in neuroendocrine cells acts as a negative regulator of secretory activity, most likely by impairing secretory granule transport.

This elegant study demonstrates that abnormal secretory activity which contributes to an increase in GH hypersecretion as the result of somatotrope hyperplasia in acromegaly has a potential molecular basis, arising from within intracellular machineries. This finding is in accordance with the low Rab18 protein content and Rab18 mRNA levels in acromegalic tissue, but not in pituitary tissue from NFPA.

Interestingly, the hypersecretory activity shown by the somatotropinoma cells has been reversed by Rab18 manipulation, opening a new therapeutic scenario aimed at control of GH hypersecretion. In this context, the family of Rab GTPases represents an attractive group of proteins that are potentially involved in the pathophysiology of several other secretory diseases since they participate in and coordinate the sequential steps of intracellular transport such as vesicle formation, motility and membrane fusion along the secretory pathway.

The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas

Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, Chahal HS, Igreja SC, Jordan S, Rowe J, Stolbrink M, Christian HC, Wray J, Bishop-Bailey D, Berney DM, Wass JA, Popovic V, Ribeiro-Oliveira A Jr, Gadelha MR, Monson JP, Akker SA, Davis JR, Clayton RN, Yoshimoto K, Iwata T, Matsuno A, Eguchi K, Musat M, Flanagan D, Peters G, Bolger GB, Chapple JP, Frohman LA, Grossman AB, Korbonits M

Department of Endocrinology, Barts and the London School of Medicine, London, UK

J Clin Endocrinol Metab 2008;93:2390–240

Background: Familial isolated pituitary adenomas (FIPA) may be associated with mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene, but the role of this molecular chaperone in pituitary tumorigenesis remains unclear.

Methods: Research of mutations and evaluation of the expression of the *AIP* sequence were performed in 26 FIPA kindreds and 85 patients with sporadic pituitary adenoma by means of studies on cell proliferation and protein–protein interaction and studies on the expression and cellular *AIP* location in both normal tissue and in adenomas.

Results: *AIP* mutations were found in 9 families. Human fibroblast, HEK293 and GH3 pituitary cell lines used to demonstrate the role of normal and mutated *AIP* molecules in reducing cell proliferation show loss of such function in *AIP* mutants. These data were confirmed by a loss of protein interaction ability of *AIP* mutants with phosphodiesterase-4A5. Immunostaining of normal pituitary tissue and of sporadic adenomas detected *AIP* expression in normal GH and prolactin cells, as well as in all types of adenomas. Electron microscopy revealed *AIP* co-localization in the secretory vesicles of normal GH cells and GH-secreting adenomas. Indeed, *AIP* was expressed in the cytoplasm of lactotroph, corticotroph and non-functioning adenomas.

Conclusion(s): *AIP* is a tumor-suppressor gene involved in familial acromegaly, where the mutations disrupt the effects of *AIP* protein. Derangement of the regulation of *AIP* protein might play a role in the etiology of sporadic adenomas.

Epidemiological evidence suggests that pituitary adenomas occur relatively frequently in the general population, with an overall rate of 1 per 1,064 cases [1]. A recent meta-analysis of the English-language literature on these tumors estimated that the overall prevalence of pituitary adenomas is 16.7% [2].

Whereas most are innocent microadenomas, pituitary adenomas occur in a familial setting in multiple endocrine neoplasia type I and in Carney's complex associated with mutations in the *MEN1* and *PRKAR1A* genes, respectively. Familial pituitary tumors account for approximately 3% of pituitary adenomas. An autosomal dominant disease with incomplete penetrance with isolated pituitary adenomas has been reported as 'familial isolated pituitary adenoma' (FIPA). Germline mutations were subsequently identified in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene. The *AIP* gene has been reported in families with GH-secreting adenomas, in families with GH- and PRL-secreting adenomas, and in families with other types of pituitary tumors as well. All types of pituitary adenomas have been found, even in families without *AIP* gene mutations. Indeed, there are now 33 different mutations (including those reported in the paper by Leontiou et al. [3]) in over 100 affected subjects [4, 5].

In addition to the six novel mutations identified in the *AIP* gene, the present study describes three major novel findings regarding the role of *AIP* in determining FIPA or sporadic adenomas. The over-expression of the wild-type *AIP* chaperon shows reduction of cell proliferation in three different cell types, including a pituitary cell line suggestive of tumor-suppressor gene properties. A second finding is the description of the cellular distribution of *AIP* in normal pituitary cells and pituitary adenomas, demonstrating that non-GH-secreting sporadic adenomas express *AIP* localized within the cytoplasm

rather than in the secretory vesicles. A third important finding is that of the first identified *AIP* promoter mutation.

A recent review [6] highlights (1) the differential diagnosis between familial pituitary adenomas and sporadic pituitary adenomas, (2) their pathogenesis, (3) clinical characteristics by tumor type, (4) genetic background, and (5) perspectives. Unraveling the genetic basis of pituitary adenomas is of great help in the elucidation of the pathogenesis of pituitary tumors. It will increase awareness of existing familial risk, which will in turn spare asymptomatic mutated subjects from late diagnoses and associated morbidities.

Pituitary-specific knockout of the Carney complex gene *Prkar1a* leads to pituitary tumorigenesis

Yin Z, Williams-Simons L, Parlow AF, Asa S, Kirschner LS

Department of Molecular Virology, Immunology, and Molecular Genetics, Ohio State University, Columbus, Ohio, USA
Mol Endocrinol 2008;22:380–387

Background: Carney complex (CNC) is a hereditary syndrome of multiple neoplasia, presenting with spotty skin pigmentation, myxomas, endocrine tumors (particularly GH-producing pituitary tumors in approximately 10% of patients), and schwannomas. The aim of this study was to evaluate the role of the CNC gene *Prkar1a* on pituitary tumorigenesis in mice.

Methods: A mouse line expressing the cre-recombinase in pituitary cells of the Pit1 lineage using rat GHRH receptor promoter (rGHRHcre) was generated. Mice carrying the rGHRHcre transgene were then crossed with mice carrying a conditional null allele of *Prkar1a*. A tissue-specific knockout of *Prkar1a* was obtained (pitKO). Cre-recombinase activity in the anterior pituitary was detected, and immunostaining, immunohistochemical characterization and hormonal profiles of pitKO tumors were determined.

Results: Tumors were observed in 30.6% of mice with a higher frequency in the pitKO mice (48%) compared to the wild-type or *Prkar1a* heterozygote mice. No difference in the occurrence of prolactinomas between pitKO and control mice was observed, while the incidence of other pituitary tumors was much higher in pitKO mice. Immunohistochemical analysis revealed a positive staining for GH, prolactin, and TSH, hormones produced by the Pit1 cell lineage. Serum GH level was significantly higher in KO than in wild-type mice, regardless of the presence or absence of tumor.

Conclusion(s): This study demonstrates, in analogy to human patients with CNC, that KO mice for *Prkar1a* are associated with the development of pituitary tumors and biochemical GH excess.

Carney complex (MIM 160980) is a rare autosomal dominant multiple endocrine neoplasia syndrome characterized by spotty skin pigmentation, cardiac and other myxomas, and different types of endocrine tumors, including multiple hyperfunctioning adrenal nodules, growth hormone-secreting pituitary tumors, and gonadal and thyroid neoplasias; rarely, malignant psammomatous melanotic schwannomas or hepatocellular carcinoma are present. The cAMP/protein kinase A (PKA; cyclic AMP (cAMP)-dependent protein kinase) pathway plays an important role in endocrine tumorigenesis, and inactivating mutations in the *PRKAR1A* gene coding for the type 1A regulatory subunit of PKA have been shown to cause the disease. Since the identification of *PRKAR1A* mutations in Carney complex, substitutions and small insertions/deletions have been found in approximately 70% of the patients. In a few families, the disease locus has been mapped to chromosome 2, but chromosome 17 could not be definitely excluded in all of them. To date, 60 different *PRKAR1A* pathogenic mutations have been described [7]. Large *PRKAR1A* deletions in the germline of 2 patients with Carney complex, both sporadic cases, have recently been reported [8].

In this study, the loss of *Prkar1a* defines a subtype of secreting adenoma with a high serum GH concentration in mice. In human beings, little is known about the genetic causes of sporadic lesions, though activating mutations of the stimulatory G protein subunit encoded by the *GNAS* gene have been described in children with McCune-Albright syndrome (for familial isolated pituitary adenoma see the paper by Leontiou et al. [3]), while inactivating mutations in *PRKAR1a* causing an increase in PKA activity and frank GH-producing pituitary adenomas are rare.

The study of pituitary tumorigenesis in pituitary-specific KO mice has advanced our knowledge of the molecular basis of pituitary tumors. Although these mice developed pituitary adenomas with moderate frequency, they represent a good model for pituitary tumorigenesis and new therapeutic developments in the human condition.

Hypothalamic and pituitary development: novel insights into the etiology

Kelberman D, Dattani MT

Developmental Endocrine Research Group, Institute of Child Health, London, UK

Eur J Endocrinol 2007;157(suppl 1):S3–S14

Background: In recent years, studies of animal models and humans affected by combined pituitary hormone deficiency (CPHD) have contributed to elucidating a complex cascade of transcription factors which participate in the control of pituitary development and function, including HESX1, PROP1, POU1F1 (Pit-1), LHX3, LHX4, SOX2 and SOX3. Mutations in these transcription factors may result in variable phenotypes ranging from isolated pituitary hormone deficiency to panhypopituitarism associated with complex disorders such as septo-optic dysplasia, holoprosencephaly and anophthalmia.

Methods: This is a review paper.

Results: The specific mutations in genes encoding for transcription factors and the resulting phenotypes are described.

Conclusion(s): Although there has been a significant increase in knowledge about the genetics of hypothalamic-pituitary development, the precise etiology of most cases of hypopituitarism remains unknown. Many genes implicated in hypopituitarism remain to be identified. The identification and characterization of novel genes will enable a greater understanding of the disorder.

In recent years, studies of animal models and humans affected by combined pituitary hormone deficiency (MPHD) have contributed to elucidating a complex cascade of transcription factors which participate in the control of pituitary development and function. At this point, a number of genes have been characterized whose loss of function causes MPHD either associated or not with extra-endocrine abnormalities. Among these are the LIM family genes, HESX1, Prop1 and Pit1, SOX2 and SOX3. The precise developmental expression of these genes has been clarified. This comprehensive review describes the specific phenotypes associated with mutations of each of these transcription factors. However, most cases of congenital CPHD do not have a clearly identifiable cause of the defect in question. Elucidating the complex hierarchy of transcription factors, which participate in the control of pituitary development, as well as their reciprocal interaction, will help to unravel the genetic basis of many undiagnosed cases.

Novel mutations in LHX3 are associated with hypopituitarism and sensorineural hearing loss

Rajab A, Kelberman D, de Castro SC, Biebermann H, Shaikh H, Pearce K, Hall CM, Shaikh G, Gerrelli D, Grueters A, Krude H, Dattani MT

Genetics Unit, DGHA, Ministry of Health, Muscat, Sultanate of Oman

Hum Mol Genet 2008;17:2150–2159

Background: Homozygous loss-of-function mutations in the transcription factor LHX3 have been associated with hypopituitarism with structural anterior pituitary defects and cervical abnormalities with or without restricted neck rotation.

Methods: Four new patients from two unrelated consanguineous pedigrees with early onset hypopituitarism and hypoglycemia were selected for the study. Three additional patients with a previous diagnosis of LHX3 deficiency were also included. Mutations in the LHX3 genes were identified by direct sequencing. In situ hybridization of human embryonic and fetal tissue was performed to investigate a possible role for LHX3 in inner ear development.

Results: Three of the 4 new patients exhibited an unusual phenotype consisting of combined pituitary hormone deficiency, severe anterior pituitary hypoplasia, skeletal abnormalities, hyperextensible joints, loose skin and sensorineural hearing loss. All patients were homozygous for an intragenic deletion of 3,088 bp resulting in complete loss of exons 2–5. The 4th patient had a similar phenotype and a homozygous transversion c.267A>T resulting in a nonsense mutation in exon 2. The other 3 patients with a pre-

vious diagnosis of Lhx3 deficiency were found to exhibit moderate and mild sensorineural hearing loss. Expression of LHX3 was observed within distinct domains of the developing inner ear in a pattern overlapping that of SOX2.

Conclusion(s): This study shows for the first time that patients with homozygous mutations in the LHX3 gene may also present with variable degrees of sensorineural hearing loss. An interaction between LHX3 and SOX2 for the development of both the inner ear and the anterior pituitary is suggested.

Mechanisms underlying pituitary hypoplasia and failed cell specification in Lhx3-deficient mice

Ellsworth BS, Butts DL, Camper SA

Department of Human Genetics, University of Michigan, Medical School, Ann Arbor, Mich., USA

Dev Biol 2008;313:118–129

Background: Normal development of the anterior pituitary depends on expression of specific transcription factors. Lhx3 is one of these transcription factors; it is first observed in the developing mouse pituitary at ED 9.5, and expression increases up to ED 12 and persists through adulthood. Mice carrying homozygous Lhx3 mutations fail to express Pit-1, do not develop differentiated gonadotropes, and show fewer corticotropes than normal. This study was performed to elucidate the role of Lhx3 on the expression of other genes involved in pituitary development.

Methods: The experiments were carried out on heterozygous Lhx3 engineered null mice. Immunohistochemistry using specific antibodies against each transcription factor of interest was performed at different embryonic ages.

Results: Null Lhx3 mice exhibited a significant increase in apoptotic cells in the ventral portion of Rathke's pouch, indicating that this phenomenon is a major contributor to the hypoplasia in Lhx3 null pituitaries. Lhx3 null pituitaries also show fewer differentiated melanotropes and corticotropes. The reduction in the number of corticotropes is due to increased apoptosis. The dying cells are limited to the regions of TPIT expression. Lhx3 deficiency also results in dorsal ectopic expression of gonadotrope-specific transcription factors, such as SF1 and ISL1, but no gonadotropin expression. This apparent abnormality in cell differentiation may be due, in part, to loss of NOTCH2, which is important for maintaining dorsal-ventral patterning in other organs.

Conclusion(s): This study has delineated the roles of Lhx3 in the differentiation of anterior and intermediate lobe cell types. Lhx3 plays a major role in pituitary development by maintaining normal dorsal-ventral patterning, cell survival, and normal expression of pituitary cell-specific transcription factors.

Several transcription factors participate in pituitary development and in the regulation of transcription of specific pituitary genes. LHX3 (also known as LIM-3) and LHX4 are members of the LIM homeodomain family of transcription factors and are essential for normal pituitary development. In a recent paper by Pfaeffle et al. [9], the authors identified three novel heterozygous missense mutations in the LHX4 gene in 5 of 253 patients with growth hormone deficiency (GHD).

Homozygous mutations in the LHX3 genes cause combined pituitary hormone deficiency with rigid cervical spine. Lhx3 null embryo mice fail to express Pit1 TPIT and NEUROD1. More recently, mutations of the LHX3 gene have also been found to be associated with milder hypopituitary phenotypes including isolated GHD. Recessive mutations in the LHX3 were detected in only 7 of 366 patients with GHD associated with one or more other deficits, and in no patients with idiopathic GHD [10]. Interestingly, limited neck rotation was present only in patients with deletion of the entire gene or with E17ter and 210V mutations.

In the present paper, Rajab et al. showed that the clinical phenotype of patients with Lhx3 deficiency can be even broader and encompass abnormal pituitary development with variable degrees of sensorineural hearing loss, as found in 7 patients with LHX3 deficiency. Overall these papers emphasize the following concepts: mutations in these two transcription factors are not a common cause of GHD, the phenotypic expression is highly variable, and the mutations are mostly found in patients with combined pituitary hormone deficiency.

The elegant paper by Ellsworth et al. explains how Lhx3 defects contribute to the development of hypopituitarism. In a series of experiments, they show that Lhx3 is essential for maintaining normal dorsal-ventral patterning, for cell survival, and for the normal expression of other transcription factors as well. Its absence leads to increased cell death. Figure 1 represent the roles of Lhx3 in the

Fig. 1. Adapted from Ellsworth et al. [13]. The roles of Lhx3 in the differentiation of anterior and intermediate lobe cell types and other factors in the initiation and maintenance of gene expression and pituitary differentiation. **a** LHX3 and LHX4 are structurally related and have redundant functions in the pituitary. ISL1 is not detected in Lhx3 null pituitaries at e12.5, but loss of Lhx4 results in an expanded pattern of ISL1 expression. **b** Tpit is required to suppress SF1 expression in the dorsal aspect of the pituitary gland. Without Tpit, SF1 is expressed in the intermediate lobe and complete gonadotrope differentiation occurs. Prop1^{df/df} mutants have no obvious abnormalities in TPIT expression, but SF1 is expressed ectopically in the dorsal aspect of the gland. SF1 is not, however, sufficient to complete the gonadotrope differentiation program in this case. NOTCH2 expression fails completely in Prop1^{df/df} mutants. Lhx3 nulls lack both TPIT and NOTCH2 and exhibit a loss of gonadotrope differentiation that appears to correlate with the lack of NOTCH2 expression. **c** Lhx3 is important for cell survival of pre-corticotropes. Lhx3 deficiency results in a severe reduction in pre-corticotropes as evidenced by reduced expression of NEUROD1 and TPIT. A reduction in differentiated corticotropes, as well as melanotopes, is demonstrated by a reduction in POMC expression. Gonadotropes are able to proceed with aspects of their differentiation process without Lhx3 as indicated by the presence of SF1, but are unable to express many markers of differentiation (FOXL2, LHβ, αGSU). Lhx3 is required for NOTCH2 expression, which may be important for appropriate dorsal-ventral cell specification and/or suppression of precocious differentiation of dorsal cell types. Finally, Lhx3 is required for expression of Pit1 and the Pit1 lineage (thyrotropes, somatotropes, lactotropes).

differentiation of anterior and intermediate lobe cell types and other factors in the initiation and maintenance of gene expression and pituitary differentiation.

These four papers make a substantial contribution to our knowledge and understanding of the development of pituitary function, as well as to the etiology and clinical presentation of congenital hypopituitarism [11].

More about pituitary signaling pathways

SOX2 plays a critical role in the pituitary, forebrain, and eye during human embryonic development

Kelberman D, de Castro SC, Huang S, Crolla JA, Palmer R, Gregory JW, Taylor D, Cavallo L, Faienza MF, Fischetto R, Achermann JC, Martinez-Barbera JP, Rizzoti K, Lovell-Badge R, Robinson IC, Gerrelli D, Dattani MT
Developmental Endocrinology Research Unit, Clinical and Molecular Genetics Unit, Institute of Child Health, London, UK
J Clin Endocrinol Metab 2008;93:1865–1873

Background: Heterozygous, de novo mutations in the transcription factor SOX2 are associated with bilateral anophthalmia or severe microphthalmia. In addition, these patients have hypopituitarism with anterior pituitary hypoplasia and gonadotropin deficiency with genital abnormalities in males. Other associated malformations include hypoplasia of the corpus callosum, hypothalamic hamartoma, hippocampal malformations, esophageal atresia, sensorineural hearing loss, and learning difficulties. This study reports the endocrine phenotype in 3 further patients with de novo heterozygous mutations in SOX2, and the expression pattern of SOX2 during human embryonic development.

Methods: Three female patients with severe eye defects and pituitary abnormalities were screened for mutations in SOX2. Chromosomal abnormalities were searched for by means of the Genome CGH Microarray technique. Intragenic mutations were identified by direct amplification and sequencing of the coding sequence of SOX2. Analysis of human embryonic/fetal material was performed by in situ hybridization and immunohistochemistry.

Results: All 3 patients harbored heterozygous SOX2 mutations: a deletion encompassing the entire gene, a novel nonsense mutation (p.Q61X), and an intragenic deletion (c.70_89del). SOX2 is expressed throughout the human brain, including the developing hypothalamus and pituitary, Rathke's pouch, and the eye. SOX2 protein inhibits β -catenin-mediated reported gene activation, and truncated mutant proteins are unable to repress this activity.

Conclusion(s): Patients with SOX2 mutations manifest a pleiotropic phenotype. This study indicates that SOX2 may play a critical role during development of the affected tissues through a failure to repress the Wnt- β -catenin pathway.

Sox2 is a member of the sex-determining region of the Y chromosome-related high mobility group box family of transcription factors. It is expressed in the developing CNS and placodes, where it plays a critical role in embryogenesis. Heterozygous mutated Sox2 mice show a reduction in size and male infertility. Conversely, heterozygous mutations in SOX2 in humans have been found to be associated with bilateral anophthalmia/microphthalmia, developmental delay, short stature, hypogonadotropic hypogonadism and male genital tract abnormalities [12].

This study confirms that SOX2 mutations can result in a pleiotropic phenotype, and suggests a molecular pathway involved in this phenomenon. In particular, in addition to the various transcription factors involved in pituitary development, WNT signaling is also emerging as an important contributor (see next article). Furthermore, several lines of investigation support roles for β -catenin which can regulate the activity of three transcription factors involved in pituitary organogenesis, including Pitx2, Nr5a1 (Sf1), and Tcf7l2 (Tcf4) [13].

WNT signaling affects gene expression in the ventral diencephalon and pituitary gland growth

Potok MA, Cha KB, Hunt A, Brinkmeier ML, Leitges M, Kispert A, Camper SA
Department of Human Genetics, University of Michigan, Ann Arbor, Mich., USA
Dev Dyn 2008;237:1006–1020

Background: Pituitary development is mediated by the temporal and spatial expression of several transcription factors in the pouch in response to different signaling pathways. The aim of this study was to evaluate how WNT signaling can affect pituitary development.

Methods: WNT knockout mice were generated and the expression of WNT pathway components was assessed.

Results: In the absence of Wnt5a, mutant mice embryos exhibit altered expression patterns of fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) in the ventral diencephalon. Wnt4-deficient mice display pituitary hypoplasia and partial loss of pituitary cell differentiation, whereas Wnt4 and Wnt5A double mutants showed a complex phenotype characterized by pituitary dysmorphology and mild hypoplasia. No pituitary malformations were documented in Wnt6 mutants.

Conclusion(s): WNT signaling regulates pituitary development through intersection with different signaling pathways.

Cell signaling through Wnt ligand-activated pathways can regulate developmental processes such as cell proliferation, differentiation, and migration. Targeted disruption of Wnt genes leads to a variety of phenotypes including defects in brain, lung, limb, and kidney development. Pituitary gland development is also dependent on the proper expression of Wnt ligands. In particular, Wnt5a regulates the shape of the pituitary gland, and Wnt4 enhances pituitary growth.

Over the last 10 years, tremendous progress has been made in understanding pituitary organogenesis. Key genes involved in pituitary development have been identified by gene mapping in humans and by targeted disruption and/or transgenic approaches in mice. Yet, many molecular signaling pathways remain unidentified. This study proves that several candidate genes downstream of WNT signaling can be discerned and that the identification of additional WNT pathway components in the pituitary gland and ventral diencephalon provides targets for investigations that will aid in fully understanding the complex network of pituitary development. These findings will help to better recognize the underlying causes of the heterogeneous condition of congenital 'idiopathic' GH deficiency, which still needs to be clarified in a large majority of cases.

Food for thought Seeking adult stem cells

Genetic approaches identify adult pituitary stem cells

Gleiberman AS, Michurina T, Encinas JM, Roig JL, Krasnov P, Balordi F, Fishell G, Rosenfeld MG, Enikolopov G
Howard Hughes Medical Institute, Department of Medicine, University of California at San Diego School of Medicine, La Jolla, Calif., USA
Proc Natl Acad Sci USA 2008;105:6332–6337

Background: Adult tissues undergo continuous cell turnover in response to stress, damage, or physiological demand. This replacement is made possible through the activity of dedicated or facultative stem cells or from self-renewing differentiated cells. The existence of multipotent stem cells in the anterior pituitary has never been demonstrated. This paper reports the existence of stem cells in the adult anterior pituitary.

Methods: Transgenic mice expressing GFP driven by regulatory elements of the nestin gene were used to identify stem cells. The expression of this transgene has been found in several types of multipotent stem cells. The development of nestin-GFP cells of the adult gland was traced during the embryonic and perinatal periods of pituitary histogenesis.

Results: GFP-positive cells were found in both the anterior and intermediate lobes of the pituitary gland. These cells expressed Lhx3, Sox2, and the epithelial markers cytokeratin 8 and EpCAM. The first nestin-GFP-expressing cells were found in Rathke's pouch at embryonic day 11.5. From embryonic day 18.5 to post-natal day 1 the number of cells gradually increased. At the end of the first postnatal week there was a second wave of pituitary growth associated with a dramatic increase in cell proliferation. At this time, a significant migration of GFP-expressing cells migrated into the gland, and among these cells a small fraction also expressed Pit-1. A series of experiments demonstrated that nestin-positive cells serve as adult stem cells; this conclusion was based on the fact that the number of GFP-positive cells in both the intermediate and anterior lobes gradually increased after birth and reached a level of 20% of all cells in the anterior pituitary by 5 months of age. GFP expression was found in all six terminally differentiated pituitary endocrine cell types. Nestin-GFP-expressing cells from the adult pituitary were put in culture and produced exponentially growing colonies which can undergo at least 25 divisions, suggesting that they may have stem cell-like properties. During exponential growth, these cells express GFP, nestin Sox2, cytokeratin 8, cytokeratin 18, Lhx3 and EpCAM. After the exponential growth phase they start to differentiate and can produce cells of all six anterior pituitary lineages. Crossing mice carrying one functional allele of the retinoblastoma Rb1 gene (Rb+/- mice) with nestin-GFP mice generated well-developed tumors in 12-month-old animals. Tumor nodules were surrounded by nestin-GFP-positive cells that expressed Lhx3, Sox2, EpCAM, and cytokeratin 8.

Conclusion(s): This study shows that the adult anterior pituitary gland contains a population of adult organ-specific multipotent stem cells. These stem cells undergo postnatal expansion and start producing differentiated cells which colonize the gland, initially populated from differentiated cells originating from embryonic precursors. These distinct cell lineages may help the maturing organism to adapt to physiological and pathological stimuli.

SOX2-expressing progenitor cells generate all of the major cell types in the adult mouse pituitary gland

Fauquier T, Rizzoti K, Dattani MT, Lovell-Badge R, Robinson IC

Division of Molecular Neuroendocrinology, Medical Research Council, National Institute of Medical Research, London, UK
Proc Natl Acad Sci USA 2008;105:2907–2912

Background: The anterior pituitary has the ability to adapt the absolute and relative number of each of its cell types in response to changing physiological demands, an ability mediated by the hypothalamus. The mechanism underlying these changes is poorly understood, although the involvement of multipotent stem cells seems plausible. Sox2, a member of the HMG transcription factors, is required for the maintenance of several stem cell populations. Heterozygous loss of SOX2 in mice and humans is associated with hypopituitarism. This study presents evidence for the presence of a small population of multipotent SOX2-positive stem cells in the adult pituitary gland.

Methods: Anterior pituitary glands from 10- to 12-week-old MF1 male mice were dissociated into single cells. Pituispheres were harvested after 5 days in culture and floating cells collected. Immunofluorescence with confocal imaging was used to identify SOX2-expressing cells.

Results: SOX 2 is expressed in embryonic pituitary progenitors and in the nonendocrine cells of the anterior pituitary. Markers of these cells are S100 protein and E-cadherin (E-cad). SOX9 expression marks differentiating progenitors in the embryo and most SOX2-positive cells in the adult. A subpopulation of SOX2-positive cells is able to generate pituispheres. Pituispheres can form from the SOX+, E-Cad+ S100– cells present in vivo in adult pituitary cells. SOX2+ progenitors are multipotent and can differentiate into each pituitary cell lineage.

Conclusion(s): This study shows that the adult anterior pituitary gland contains a small subpopulation of SOX2+ cells that are able to form pituispheres which can proliferate and differentiate into all pituitary cell types.

Research on stem cells has considerably increased over the past few years. Recognition of stem cells' potential usefulness in tissue repair and regeneration has opened up a new field of research which is rapidly expanding. The first study shows, for the first time, that the adult pituitary gland contains a population of stem cells that are different from embryonic precursors. These cells possess all the characteristics of multipotent stem cells, including the potential to differentiate into tumor cells. Future research on pituitary stem cells will help to clarify the precise mechanism of pituitary-cell-lineage differentiation and pituitary tumorigenesis.

Another exciting aspect to be explored is the potential role of multiprogenitor cells for replacing pituitary cell lineages. Indeed, the results suggest a novel way that the pituitary gland may adapt postnatally, even in adolescents and adults to traumatic stress or to normal life changes like pregnancy. Certainly, multipotential progenitor cells residing in the subcortical white matter of the adult human brain have recently been identified as having the potential to replace neuronal lineages [14].

Another exciting finding is illustrated in the paper by Gleiberman et al. who provide evidence of the persistence of SOX2+ cells in the adult pituitary gland that are capable of differentiating into all pituitary cell types. Future in vitro manipulation will be extremely helpful in order to understand the mechanisms underlying the differentiation and remodeling of this progenitor population.

Important for clinical practice No alternative to prevention

Traumatic brain injury is a rarely reported cause of growth hormone deficiency

McDonald A, Lindell M, Dunger DB, Acerini CL

Department of Pediatrics, University of Cambridge, Cambridge, UK

J Pediatr 2008;152:590–593

Background: Traumatic brain injury (TBI) is a major health problem worldwide, and an association between TBI and long-term hypothalamic dysfunction has long been recognized. Recent retrospective studies have shown a prevalence of anterior pituitary deficiency of between 23 and 69% in adult TBI survivors. The aim of this study was to determine the frequency of TBI in patients with growth hormone deficiency (GHD) from a large GHD-patient database.

Methods: Data were retrieved from the Pfizer International Growth Study (KIGS) database.

Results: A total of 23,863 patients were identified with GHD due to either idiopathic GHD (IGHD; n = 23,722) or TBI (n = 141). 38.2% of MRI scans were abnormal in the TBI patients compared to none in the IGHD patients. Multiple pituitary hormone deficiencies (two or more) were significantly more frequent in the TBI patients. Patients with TBI-induced GHD had a better response to the first year of GH treatment.

Conclusion(s): The authors concluded that clinicians need to be made increasingly aware of TBI as a possible cause of hypopituitarism. Prospective longitudinal studies to explore the nature and frequency of TBI-induced GHD are recommended.

Pituitary function in pediatric survivors of severe traumatic brain injury

Poomthavorn P, Maixner W, Zacharin M

Department of Endocrinology and Diabetes, Royal Children's Hospital, Melbourne, Australia

Arch Dis Child 2008;93:133–137

Background: TBI-induced hypopituitarism among adult survivors has been increasingly studied in recent years. This study aimed to identify pituitary dysfunction in a cohort of surviving pediatric patients after a severe TBI.

Methods: The study consisted of patients with severe TBI admitted to the Royal Children's Hospital of Melbourne, Australia, between January 1995 and June 2005. Patients were selected if they had had a severe TBI, had CT evidence of brain injury and were discharged alive from the hospital. A total of 117 patients who had suffered a severe TBI were identified, 4 of whom had been diagnosed with anterior pituitary hormone deficiencies prior to the injury. Fifty patients (31 males and 19 females, age 4.3–21.3 years) agreed to enroll in the study. The TBI had occurred at ages ranging from 0.25 to 16.80 years and the time from TBI to study ranged from 0.9 to 8.5 years. Weight, height, BMI, and pubertal stage were assessed. Baseline hormonal investigation was carried out including measurement of fT4, TSH, IGF-I, IGFBP3, LH, FSH, morning cortisol, testosterone, estradiol and prolactin. Serum sodium and osmolality

were also measured. In patients with poor growth velocity and low IGF-I, a glucagon stimulation test was performed for GH and cortisol determination.

Results: Evidence of pituitary dysfunction was found in 9 patients, 4 of whom had clinically manifested the dysfunction prior to the study. All 4 had combined pituitary hormone deficiency, including GHD. Among the other 5 patients, 1 had precocious puberty, 3 had mild isolated corticotrope dysfunction and 1 had central hypothyroidism.

Conclusion(s): This study confirms that children who suffer a TBI may develop pituitary dysfunction and, therefore, pituitary function should be controlled in these patients.

TBI has long been recognized as a possible cause of pituitary dysfunction, with the first description dating back to the 1940s. In the last decade, a number of studies have shown that adults who suffered TBI in childhood may develop anterior pituitary hormone deficiency with a reported frequency of between 23 and 69%. In recent years, studies in children have confirmed that TBI can cause pituitary dysfunction in as many as 10% of children who suffer a TBI. These two studies add further information to the current literature by showing that TBI can cause hypothalamic-pituitary dysfunction in children, albeit with a frequency that is somewhat lower than that reported in adults. Interestingly, there does not seem to be any correlation between the severity of the TBI and the occurrence of anterior pituitary dysfunction. Basal skull fracture, with incarceration of the pituitary gland, is more frequently found among patients with pituitary dysfunction. Unrecognized pituitary dysfunction after TBI can adversely affect the outcome of children and adolescents, not only in terms of growth and development, but also in terms of survival probability. While central diabetes insipidus represents an acute feature and sometimes the symptoms and signs may be transient, ACTH deficiency should be ruled out and the ACTH axis needs to be more stringently evaluated in the short-term period. Short- and long-term follow-ups of TBI-affected children are therefore recommended since TBIs should be considered a possible cause of hypopituitarism in children.

CNS infections – Harmful for pituitary?

Hypothalamic-pituitary insufficiency following infectious diseases of the central nervous system

Schaefer S, Boegershausen N, Meyer S, Ivan D, Schepelmann K, Kann PH
Division of Endocrinology and Diabetology, Philipps University Hospital, Marburg, Germany
Eur J Endocrinol 2008;158:3–9

Background: Infectious diseases of the central nervous system (CNS) may affect hypothalamic-pituitary function. The aim of this study was to evaluate the incidence of endocrine dysfunction in subjects who had experienced an acute CNS infection.

Methods: An insulin tolerance test was performed in 19 subjects previously affected by neuroborreliosis, encephalitis, or meningitis, 10–56 (mean 26.1 ± 13.1) months following an acute infection.

Results: The insulin tolerance test identified 4 patients (21%; 2 males, 2 females) with central isolated adrenal insufficiency (peak cortisol $<181.25 \mu\text{g/l}$). Borderline hypogonadotropic hypogonadism was found in 2 (11%) male patients (basal testosterone between 2.4 and 3.0 mg/l), while no other pituitary dysfunctions (including diabetes insipidus and/or hyperprolactinemia) were found in the other patients.

Conclusion(s): Hypothalamic-pituitary dysfunction and especially isolated central adrenal insufficiency in the long term can complicate about 30% of cases of CNS infections.

Hypothalamic-pituitary dysfunction after TBI, subarachnoid hemorrhage and ischemia has been underestimated for a long time. This paper raises an additional concern about pituitary hormone defects by focusing on the relationship between CNS infections and pituitary involvement.

HIV-infected patients display a wide range of pituitary defects and several other viral infections play a role in pituitary diseases encompassing a wide spectrum of pathologies including autoimmune (lymphocytic) hypophysitis, granulomatous hypophysitis, local manifestations of systemic disease, and a

multitude of infectious processes. In addition, the IGF system is affected in patients with viral infections (herpes simplex virus, cytomegalovirus, rotavirus, and adenovirus). Thus CNS infections mediated by herpes viruses, paramyxoviruses and other viruses might affect pituitary function in susceptible children and adolescents who have not undergone vaccination. Greater awareness of this possible complication and appropriate testing are encouraged. This very small study of a common disease is but a call for others to run a large scale one.

Copeptin – Better than vasopressin

Anterior and posterior pituitary function testing with simultaneous insulin tolerance test and a novel copeptin assay

Katan M, Morgenthaler NG, Dixit KC, Rutishauser J, Brabant GE, Muller B, Christ-Crain M

Department of Endocrinology, University Hospitals, Basel, Switzerland

J Clin Endocrinol Metab 2007;92:2640–2643

Background: The water deprivation test is usually performed in order to differentiate between diabetes insipidus and primary polydipsia in subjects presenting with polyuria and polydipsia. The water deprivation test is cumbersome and an alternative non-osmotic stimulus is advisable. Because plasma arginine vasopressin (AVP) measurement is difficult, a new assay for the measurement of copeptin has recently been introduced. The aim of this study was to evaluate copeptin as a marker for the diagnosis of disorders of both the posterior and anterior pituitary during insulin tolerance test (ITT).

Methods: 38 patients (9 affected with central diabetes insipidus, 29 with normal posterior pituitary function) underwent ITT, principally to study anterior pituitary function. Copeptin determination was performed 0, 30, 45 and 90 min after intravenous insulin injection, using a new sandwich immunoassay.

Results: Basal copeptin levels were 3.7 ± 1.5 pM, with a peak of 11.1 ± 4.6 pM at time +45 min in subjects without central diabetes insipidus. In affected patients, basal and stimulated copeptin levels, 2.4 ± 0.5 and 3.7 ± 0.7 pM, respectively, were significantly lower. A study of the diagnostic accuracy of the results indicates 4.75 pM as the cutoff value that distinguishes between subjects with central diabetes insipidus and those with normal posterior pituitary function.

Conclusion(s): Copeptin evaluation during ITT is potentially useful for the evaluation of both posterior and anterior pituitary function.

AVP derives from a precursor protein, pre-pro-vasopressin, which consists of a signal peptide, AVP, neurophysin II, and copeptin. AVP is a key regulator of water balance, but its instability and short half-life makes reliable measurement difficult and precludes routine use. A new immunoluminometric assay (using 2 polyclonal antibodies to amino acids 132–164 of pre-pro-vasopressin) to quantify AVP release by using copeptin, a glycopeptide comprising the C-terminal part of the AVP prohormone, has recently been developed [15]. Copeptin, together with vasopressin, is co-secreted from the posterior pituitary and therefore mirrors the amount of vasopressin in the circulation. It has been shown to be stable for days after blood withdrawal and is quickly and easily measurable, suggesting qualities that make it a viable alternative to AVP. Copeptin reacts similarly to AVP in the presence of disorders affecting water homeostasis. Moreover, involvement of both posterior pituitary and anterior pituitary function is not uncommon in patients with pituitary tumors or in lymphocytic-infundibulohypophysitis. Consequently, the ability of ITT to simultaneously stimulate the vasopressin/copeptin system and the GH/ACTH axis could be very useful in the evaluation of patients with traumatic or functional section of the pituitary stalk. The measurement of neurophysin II levels would hold the same potential benefit compared with copeptin, yet, has not reached the point of clinical use. A recent review provides very useful information about the clinical use of copeptin [16].

Treatment of ACTH-dependent Cushing's syndrome: a consensus statement

Billor BM^a, Grossman AB^b, Stewart PM^c, Melmed S^d, Bertagna X^e, Bertherat J^e, Buchfelder M^f, Colao A^g, Hermus AR^h, Hofland LJⁱ, Klibanski A^j, Lacroix AJ^k, Lindsay JK^l, Newell-Price J^l, Nieman LK^m, Petersenn Sⁿ, Sonino N^o, Stalla GK^p, Swearingen B^q, Vance ML^q, Wass JA^r, Boscaro M^s

^aNeuroendocrine Clinical Center, Massachusetts General Hospital, Boston, Mass., USA; ^bDepartment of Endocrinology, St Bartholomew's Hospital, London, UK; ^cDivision of Medical Sciences, University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK; ^dDepartment of Medicine, Cedars Sinai Medical Center, Los Angeles, Calif., USA; ^eEndocrinology Department, Hôpital Cochin, AP-HP, Faculté de Médecine Paris Descartes, Paris, France; ^fNeurochirurgische Klinik (M.B.), Universität Erlangen, Nürnberg, Germany; ^gDipartimento di Endocrinologia ed Oncologia Molecolare e Clinica, Università degli Studi di Napoli Federico II, Napoli, Italy; ^hDepartment of Endocrinology, University Medical Center Nijmegen, The Netherlands; ⁱDepartment of Internal Medicine, Division of Endocrinology, Erasmus Medical Center, Rotterdam, The Netherlands; ^jDivision of Endocrinology, Department of Medicine, Centre Hospitalier de l'Université de Montréal, Montréal, Québec, Canada; ^kAltnagelvin Area Hospital, Londonderry, UK; ^lThe Medical School, University of Sheffield, Sheffield, UK; ^mReproductive Biology and Medicine Branch, National Institutes of Health, Bethesda, Md., USA; ⁿDivision of Endocrinology, University of Essen, Essen, Germany; ^oDepartment of Statistical Sciences, University of Padova, Padova, Italy; ^pDepartment of Endocrinology, Max Planck Institute of Psychiatry, Munich, Germany; ^qUniversity of Virginia Health System, Charlottesville, Va., USA; ^rDepartment of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK; ^sDivision of Endocrinology, Institute of Internal Medicine, Polytechnic University of Marche, Ancona, Italy
J Clin Endocrinol Metab 2008 Apr 15. [Epub ahead of print]

Background: The management of ACTH-dependent Cushing's syndrome is challenging and there is a need for a consensus on the management of this rare disorder.

Methods: Thirty-two leading experts in the management of ACTH-dependent Cushing's syndrome met in order to tackle (1) criteria for cure and remission; (2) surgical treatment; (3) treatment options in persistent disease after transsphenoidal surgery; (4) medical treatment, and (5) management of ectopic ACTH syndrome and Nelson's syndrome.

Results: Participants agree that early diagnosis and prompt therapy is warranted, and that the first-line treatment for ACTH-dependent Cushing's syndrome is curative surgery whenever possible. Second-line treatments include more radical surgery, radiation therapy (for Cushing's disease), medical therapy and bilateral adrenalectomy.

Conclusion(s): ACTH-dependent Cushing's syndrome is a heterogeneous disorder requiring a multidisciplinary and individualized approach to patient management.

Cushing's syndrome is rare in childhood and adolescence compared to other endocrine diseases. ACTH-secreting adenomas are the most common cause of adrenal hyperfunction and the most frequently occurring pituitary adenomas after prolactinomas.

The clinical presentation in the pediatric population is highly variable, with signs and symptoms that can range from very subtle to specific and obvious. The diagnosis is generally delayed by more than 2 years since a decrease in growth rate may be the only symptom over a long-term period. The differential diagnosis of Cushing's syndrome includes adrenal tumors, ectopic ACTH production, and ectopic CRH-secreting tumors. 'The Endocrine Society Clinical practice guidelines' for the diagnosis of Cushing's syndrome and systematic review meta-analyses have recently been published [17, 18].

Moreover, the management of Cushing's syndrome is well covered by this consensus article which provides comprehensive recommendations for this rare disorder. Although the implications for practice and research are markedly evident, the guideline panels will require evidence that patients benefit in important ways if they are diagnosed when the disease is subtle and mild rather than when it is florid and severe. This review covers childhood Cushing's only superficially, and this topic deserves attention.

Hes1 and Hes5 control the progenitor pool, intermediate lobe specification, and posterior lobe formation in the pituitary development

Kita A, Imayoshi I, Hojo M, Kitagawa M, Kokubu H, Ohsawa R, Ohtsuka T, Kageyama R, Hashimoto N

Department of Neurosurgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

Mol Endocrinol 2007;21:1458–1466

Background: The anterior pituitary and the posterior pituitary develop from two different sources.

The posterior pituitary is formed from the infundibulum by downward evagination of neural tissue from the floor of the third ventricle. Hes genes, known as Notch effectors, play a crucial role in specifying cellular outcomes during the development of various tissues and organs and are essential for the maintenance of neural stem cells.

Methods: Hes1, Hes5 and Hes-1 and Hes5-double mouse mutants were generated. In situ hybridization and immunostaining studies were then performed.

Results: Hes1 and Hes5 mutants showed severe pituitary hypoplasia caused by acceleration of pituitary progenitor cell differentiation and lack of the intermediate lobe. Double mutant mice showed a more severe phenotype, with anterior pituitary hypoplasia and agenesis of the posterior lobe. The latter was probably caused by abnormal evagination of the diencephalon.

Conclusion(s): Hes genes play various roles in pituitary embryogenesis in mice. Hes1 and Hes5 gene mutations may be responsible for pathological phenotypes involving the anterior, intermediate and posterior pituitary lobes.

The molecular mechanisms and roles of transcription factors in anterior pituitary development are now much clearer, both in animals and humans, but little is known about the mechanisms that regulate posterior pituitary development.

Early pituitary development occurs in three steps, the first being the formation of a thickening (placode) in the oral ectoderm in response to signaling molecules from the diencephalon. The second step is the formation of a definitive Rathke's pouch, again triggered by a signal from the diencephalon. In the third step, oral ectoderm cells in the pouch differentiate into various pituitary cell types to form a nascent anterior pituitary gland and pars intermedia.

Hypothalamo-neurohypophysis consists of magnocellular neurons producing the peptide hormones vasopressin or oxytocin. The cell bodies are located in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) in the hypothalamus, and axons project to the neurohypophysis, where the hormones are secreted into the blood stream. The early differentiation of these cell lineages has recently become much more comprehensible through the clarification of the role of transcription factors in hypothalamic development. The *Slm1*, *ARNT2*, *OTP*, *BRN2* genes appear to be involved in the cascade of transcription factors implicated in the development of the neuroendocrine hypothalamus leading to the completion of posterior pituitary development by the end of the first trimester, when vasopressin and oxytocin can be detected in neurohypophyseal tissue. In particular, null mutation of *UNCx4.1* showed a phenotype consisting of an ectopic localization that extends vasopressinergic axons from PVN and SON, which no longer halt at the proper position in the neurohypophysis, but instead grow into the anterior pituitary lobe [19]. In humans, a similar picture has been reported in association with cerebral malformations [20].

Hes1 and Hes5 are essential effectors for Notch signaling that regulate the maintenance of progenitor cells and the timing of their differentiation in various tissues and organs and are essential for the regulation of neural stem cells. In the nervous system, both *Hes1* and *Hes5* are essential for the regulation of neural stem cells, while in the endocrine system, *Hes1* controls pancreatic cell differentiation: indeed, an analysis of mice deficient for *Prop1* has indirectly implicated involvement of the Notch pathway in pituitary development.

In this study, a Hes1-null pituitary gland was reduced in size but was otherwise morphologically normal compared with the control. Indeed, in Hes1-Hes5 double-mutant mice, the evagination of the infundibulum was affected and the neurohypophysis was lost compared to both the wild-type and Hes1-null mice, suggesting that both Hes genes are essential for the formation of the neurohypophysis.

What can we learn from rodents about prolactin in humans?

Ben-Jonathan N, LaPensee CR, LaPensee EW

Department of Cell and Cancer Biology, University of Cincinnati, Cincinnati, Ohio, USA

Endocr Rev 2008;29:1–41

Prolactin (PRL) is a 23-kDa protein hormone that binds to a single-span membrane receptor, a member of the cytokine receptor superfamily, and exerts its action via several interacting signaling pathways. PRL is a multifunctional hormone that affects multiple reproductive and metabolic functions and is also involved in tumorigenicity. In addition to being a classical pituitary hormone, PRL in humans is produced by many tissues throughout the body where it acts as a cytokine. The objective of this review is to compare and contrast multiple aspects of PRL, from structure to regulation, and from physiology to pathology in rats, mice, and humans. At each juncture, questions are raised whether, or to what extent, data from rodents are relevant to PRL homeostasis in humans. Most current knowledge on PRL has been obtained from studies with rats and, more recently, from the use of transgenic mice. Although this information is indispensable for understanding PRL in human health and disease, there is sufficient disparity in the control of the production, distribution, and physiological functions of PRL among these species to warrant careful and judicious extrapolation to humans.

PRL is a 23-kDa protein hormone; its membrane receptor belongs to the cytokine receptor superfamily and acts via several signal transduction mechanisms. PRL functions extend from lactation induction to metabolic functions and finally to tumorigenicity. An important difference between PRL and other pituitary hormones is its production by many body tissues, with significant cytokine-like actions. The regulation of pituitary PRL production/release is more complex and centralized in rodents than in humans. In fact, many of the controls of PRL in humans have shifted from a central site to the periphery. At each extrapituitary site, PRL is independently regulated by local factors and acts as an autocrine/paracrine cytokine contributing minimally to circulating PRL levels in humans. In this respect, rodents cannot serve as an appropriate model.

The participation of PRL in the reproductive process is highly species-specific and its role in human reproduction, with the exception of lactation, remains enigmatic.

More is known about PRL/PRL receptor (PRLR) variants in humans than in rodents, where the importance of modifications such as phosphorylation, glycosylation, cleavage, or oligomerization under *in vivo* conditions are well recognized. Another important aspect of PRL which has been overlooked for a long time is their metabolic functions. Cell lines that express the PRLR provide an excellent opportunity to study interactions between PRL and hormones such as insulin, glucocorticoids, and catecholamines that affect adipogenesis, glucose, and lipid metabolism. Whether PRL is involved in human obesity and in insulin resistance by means of its capacity to alter the production and release of adipokines such as leptin, adiponectin, and IL-6 remains a subject for future research.

This review provides detailed information on PRL physiology and pathophysiology in animals and humans. Most current PRL data have been obtained from studies of animals and, especially in recent years, from transgenic mice. The pituitary and extrapituitary roles of PRL are discussed and awareness of the versatility and adaptive nature of PRL deserve careful extrapolation from studies of different species to human beings. Can we learn from rodents about PRL in humans? The answer is that although some features of PRL and its action are similar among species, many are not. For more details you can refer to the review.

References

1. Daly AF, Rixhon M, Adam C, Dempegioti A, Tichomirowa MA, Beckers A: High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. *J Clin Endocrinol Metab* 2006;91:4769–4775.
2. Ezzat S, Asa SL, Couldwell WT, Barr CE, Dodge WE, Vance ML, et al: The prevalence of pituitary adenomas: a systematic review. *Cancer* 2004;101:613–619.
3. Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, et al: The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. *J Clin Endocrinol Metab* 2008;93:2390–2401.

4. Maghnie M, Secco A, Loche S: Pituitary; in Carel JC, Hochberg Z (eds): Yearbook of Pediatric Endocrinology. Basel, Karger, 2007, pp 6–7.
5. Maghnie M, Secco A, Loche S: Pituitary; in Carel JC, Hochberg Z (eds): Yearbook of Pediatric Endocrinology. Basel, Karger, 2007, pp 11–12.
6. Beckers A, Daly AF: The clinical, pathological, and genetic features of familial isolated pituitary adenomas. *Eur J Endocrinol* 2007;157:371–382.
7. Nadella KS, Jones GN, Trimboli A, Stratakis CA, Leone G, Kirschner LS: Targeted deletion of Prkar1a reveals a role for protein kinase A in mesenchymal-to-epithelial transition. *Cancer Res* 2008;68:2671–2677.
8. Horvath A, Bossis I, Giatzakis C, Levine E, Weinberg F, Meoli E, et al: Large deletions of the PRKAR1A gene in Carney complex. *Clin Cancer Res* 2008;14:388–395.
9. Pfaeffle RW, Hunter CS, Savage JJ, Duran-Prado M, Mullen RD, Neeb ZP, et al: Three novel missense mutations within the LHX4 gene are associated with variable pituitary hormone deficiencies. *J Clin Endocrinol Metab* 2008;93:1062–1071.
10. Pfaeffle RW, Savage JJ, Hunter CS, Palme C, Ahlmann M, Kumar P, et al: Four novel mutations of the LHX3 gene cause combined pituitary hormone deficiencies with or without limited neck rotation. *J Clin Endocrinol Metab* 2007;92:1909–1919.
11. Maghnie M, Secco A, Loche S: Pituitary; in Carel JC, Hochberg Z (eds): Yearbook of Pediatric Endocrinology. Basel, Karger, 2005, pp 10–11.
12. Maghnie M, Secco A, Loche S: Pituitary; in Carel JC, Hochberg Z (eds): Yearbook of Pediatric Endocrinology. Basel, Karger, 2007, pp 12–13.
13. Ellsworth BS, Butts DL, Camper SA: Mechanisms underlying pituitary hypoplasia and failed cell specification in Lhx3-deficient mice. *Dev Biol* 2008;313:118–129.
14. Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G 2nd, Jiang L, et al: Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. *Nat Med* 2003;9:439–447.
15. Morgenthaler NG, Struck J, Alonso C, Bergmann A: Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006;52:112–119.
16. Morgenthaler NG, Struck J, Jochberger S, Dunser MW: Copeptin: clinical use of a new biomarker. *Trends Endocrinol Metab* 2008;19:43–49.
17. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al: The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2008;93:1526–1540.
18. Elamin MB, Murad MH, Mullan R, Erickson D, Harris K, Nadeem S, et al: Accuracy of diagnostic tests for Cushing's syndrome: a systematic review and metaanalyses. *J Clin Endocrinol Metab* 2008;93:1553–1562.
19. Asbreuk CH, van Doorninck JH, Mansouri A, Smidt MP, Burbach JP: Neurohypophysial dysmorphogenesis in mice lacking the homeobox gene *Uncx4.1*. *J Mol Endocrinol* 2006;36:65–71.
20. Mitchell LA, Thomas PQ, Zacharin MR, Scheffer IE: Ectopic posterior pituitary lobe and periventricular heterotopia: cerebral malformations with the same underlying mechanism? *AJNR Am J Neuroradiol* 2002;23:1475–1481.