REVIEW

Cell cycle control of pituitary development and disease

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Abstract

The pituitary gland regulates diverse physiological functions, including growth, metabolism, reproduction, stress response, and ageing. Early genetic models in the mouse taught us that the pituitary is highly sensitive to genetic alteration of specific cell cycle regulators such as the retinoblastoma protein (pRB) or the cell cycle inhibitor $p27^{Kip1}$. The molecular analysis of human pituitary neoplasias has now corroborated that cell cycle deregulation is significantly implicated in pituitary tumorigenesis. In particular, proteins involved in cyclin-dependent kinase regulation or the pRB pathway are altered in nearly all human pituitary tumors. Additional cell cycle regulators such as PTTG1/securin may have critical roles in promoting genomic instability in pituitary neoplasias. Recent experimental data suggest that these cell cycle regulators may have significant implications in the biology of putative progenitor cells and pituitary homeostasis. Understanding how cell cycle regulation controls pituitary biology may provide us with new therapeutic approaches against pituitary diseases.

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Introduction

The pituitary gland is a central endocrine organ that regulates basic physiological functions including growth, reproduction, and metabolic homeostasis. The mammalian pituitary is composed of three lobes: the posterior pituitary (PP), the intermediate lobe (IL, atrophic in humans), and the anterior pituitary (AP). The versatile endocrine functions of the gland are carried out by six cell types residing in the AP and IL of the pituitary gland. These cell types are defined by the hormone they produce and secrete: corticotropes producing ACTH, thyrotropes secreting TSH, somatotropes secreting GH, lactotropes that produce prolactin, gonadotropes secreting LH, and FSH, and the IL-specific melanotropes secreting MSH (Fig. 1). The adult pituitary arises from progenitors of a neuroectodermic primordium known as Rathke's Pouch in a temporal and spatial-specific fashion during pituitary development (Melmed 2003, Zhu *et al.* 2007). By embryonic day (E)9·5, specific signaling gradients induce the formation of the Rathke's Pouch from the oral ectoderm. The major proliferation phase and the positional determination and lineage commitment of the pituitary take place by mid-gestation (E11·5–E13·5) and the gland is not terminally differentiated till birth. Major pathways implicated in the development of the pituitary include the Notch and Wnt regulatory networks, which are mainly active in the early phases of pituitary organogenesis and are essential for the emergence of somatotropes, lactotropes, and thyrotropes (Zhu *et al.* 2007). The regulation of the proliferative ability of pituitary cells in adulthood is not well established, although different classes of stem/progenitor cells have been postulated (Vankelecom 2007). A side population that efficiently excludes the Hoechst 33342 dye has been shown to segregate with sphere-forming cells in the pituitary (Chen *et al.* 2005). Pituitary colony-forming cells that display notable clonogenic potential have also been isolated (Lepore *et al.* 2005). More recently, stem-cell specific markers such as SOX2+, SOX9, or OCT4 in addition to other epithelial markers have been found in a single-cell layer in the marginal zone suggesting the presence of stem/progenitor cells that may contribute to cell renewal in the adult pituitary (Fauquier *et al.* 2008, Garcia-Lavandeira *et al.* 2008, Gleiberman *et al.* 2008).

Control of the cell cycle by cyclin-dependent kinases and their regulators

The cell cycle is the process by which cells divide into daughter cells. Cell division is traditionally divided into four phases: S phase (synthesis of DNA) in which is produced the duplication of the genome, M phase

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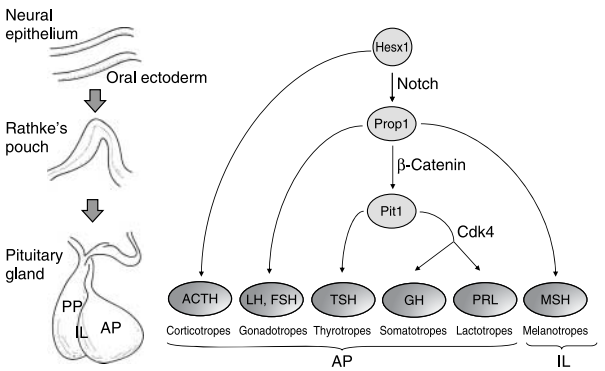

Figure 1 Development of pituitary and generation of hormone-producing cells from progenitors. Some representative transcription factors and signaling pathways are indicated. The cell cycle regulator CDK4 may be involved in the post-natal production of some AP cells such as somatotropes and lactotropes. The requirement for CDK4 in other pituitary cells is not clear as the whole pituitary is smaller in Cdk4-null mice. PP, posterior pituitary; IL, intermediate lobe; and AP, anterior pituitary.

(mitosis) in which the genetic material is segregated into two identical daughter cells, and two phases of growing and transition, called G (gap) phases (Fig. 2). G1 phase occurs before S phase; and G2 precedes mitosis. In mammalian cells, this process is driven by several protein kinases that regulate progression through the various phases of the cell cycle. Among these kinases, cyclin-dependent kinases (CDKs) are critical regulators of the transition through the different phases of the cell cycle (Malumbres & Barbacid 2005). CDK activity is modulated by fluctuations in the cellular concentration of their activators (cyclins) or inhibitors (CDK inhibitors or CKIs), which are regulated by specific transcriptional induction by mitogenic and anti-mitogenic pathways and proteolysis by the ubiquitin-proteosome system. A variety of cyclin and CDK complexes participate in the regulation of G1/S or G2/M transitions. D-type cyclins (D1, D2, and D3) act as sensors of multiple mitogenic signals to activate CDK4 and CDK6 and to facilitate the progression during G1. CDK2–cyclin E (E1 and E2) complexes become active at the end of G1 and participate in the transition from G1 to S phase. E-type cyclins are substituted by A-type cyclins (A1, A2) to activate CDK2 and CDK1 at the end of S phase and during G2. Finally, the mitotic complex formed of CDK1–cyclin B (mostly B1 and B2) is involved in the progression through G2 and entry into M phases.

The specific inhibitors of CDKs (CKIs) also play a major role in the cell cycle as mediators of antimitogenic signals or checkpoint responses. They counteract CDK function, either by blocking their activation, or by impairing substrate/ATP access. There are two families of CKIs, the INK4 family and the Cip/Kip family. The INK4 family ($p16^{INK4a}$, $p15^{INK4b}$, $p18^{INK4c}$, and $p19^{INK4d}$) inhibits progression through G1/S by binding CDK4 and CDK6. By contrast, members of the Cip/Kip family ($p21^{Cip1}$, $p27^{Kip1}$,and $p57^{Kip2}$) have different roles depending on the CDK–cyclin complex they bind to. Association to CDK2 and CDK1 complexes blocks their kinase activity, whereas the role of Cip or

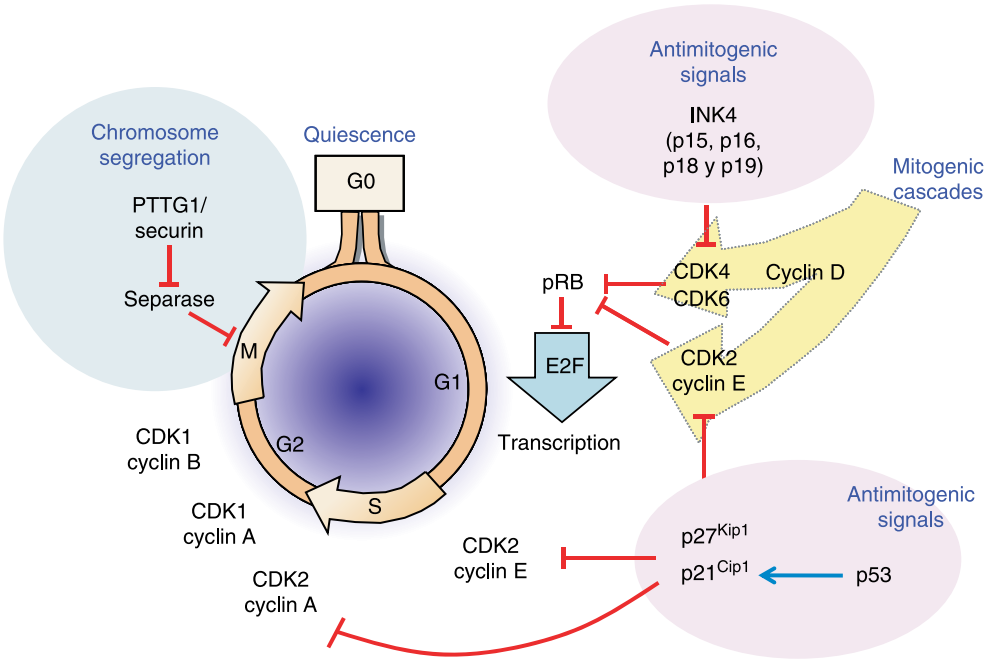

Figure 2 Control of the cell division cycle by major regulators involved in pituitary biology. S, DNA synthesis; M, mitosis; G1 and G2 correspond to 'gap' phases. Quiescence is frequently referred to as G0.

Kip binding to CDK4–cyclin D or CDK6–cyclin D complexes is unclear (Malumbres & Barbacid 2005).

The primary substrates of the CDKs in G1 progression are the members of the retinoblastoma protein family (pRB). pRB negatively regulates entry into the cell cycle and G1/S progression (Malumbres & Barbacid 2001). pRB binds to the transcription factor family E2F to target cell cycle-specific genes for repression. In non-cycling cells, pRB is hypo-phosphorylated and this active form is able to repress cell cycle progression. CDK–cyclin mediated phosphorylation of pRB provokes its release from E2F factors that are then active to induce the expression of cell cycle genes required for S and M phases.

Pituitary function and mouse models of cell cycle deregulation

Little is known about the implication of cell cycle regulators in pituitary gland development. However, in the last years, several mouse models of cell cycle regulators, such as pRB, CDKs, or CKIs, have suggested that some endocrine tissues such as the pituitary gland are critical targets of cell cycle deregulation in cancer and other diseases.

The initial link between cell cycle regulation and the pituitary comes from the seminal genetic analysis of pRB in the mouse (Clarke *et al.* 1992, Jacks *et al.* 1992, Lee *et al.* 1992). In contrast to humans, in whom individuals who inherit one defective copy of pRB gene have a roughly 90% likelihood of developing retinoblastoma at an early age (Matsunaga 1980), mice heterozygous for pRB did not develop retinoblastoma but instead developed pituitary tumors by the age of 12 months (Jacks *et al.* 1992; Table 1). Tumor incidence and histological phenotype of the tumors was highly dependent on the mouse strain suggesting additional modifier genes in pituitary tumor development (Leung *et al.* 2004). Tumor incidence provoked by the partial deletion of pRB is partially reverted by a mutation in pRB effectors such as E2f1 (Yamasaki *et al.* 1998) or E2f4 (Lee *et al.*

Table 1 Mouse models of cell cycle-related proteins involved in pituitary biology

Model	Pituitary phenotype	Incidence (%)	Latency (months)	References
Pituitary hyperplasia				
$pRb^{+/-}$	IL tumors	100	16	Jacks *et al.* (1992)
$pRb^{+/-}$; E2f-$1^{-/-}$	IL tumors. Decreased versus pRB mutants	62	18	Yamasaki *et al.* (1998)
$pRB^{+/-}$; E2f-$4^{-/-}$	IL tumors. Decreased versus pRB mutants	78	20	Lee *et al.* (2002)
$p27^{-/-}$	IL tumors	100	12	Kiyokawa *et al.* (1996) and Nakayama *et al.* (1996)
$p27^{CK-/CK-}$	IL tumors	75	10·7	Besson *et al.* (2007)
$p27^{-/-}$;$Cdk2^{-/-}$	IL tumors. No differences versus P27 $^{-/-}$;$Cdk2^{+/+}$	100	12	Martin *et al.* (2005)
$pRb^{+/-}$; p27 $^{-/-}$	Cooperation in IL tumors	90	7	Park *et al.* (1999)
$p18^{-/-}$	Tumors in IL and AP	50	15	Franklin *et al.* (1998)
$p16^{-/-}$; $p18^{-/-}$	IL tumors. Shorter latency versus p18 mutants	50	10	Ramsey *et al.* (2007)
$p15^{-/-}$; $p18^{-/-}$	No differences versus p18 mutants	50	15	Latres *et al.* (2000)
$p19^{-/-}$; $p18^{-/-}$	No differences versus p18 mutants	50	15	Zindy *et al.* (2001)
$p21^{-/-}$; $p18^{-/-}$	Cooperation in IL tumors	90	13	Franklin *et al.* (2000)
$p27^{-/-}$; $p18^{-/-}$	IL and AP undifferentiated tumors	100	3·5	Franklin *et al.* (1998)
$pRB^{+/-}$; $p21^{-/-}$	IL tumors	100	12	Brugarolas *et al.* (1998)
$Cdk4^{R24C/R24C}$	AP tumors	25	15	Rane *et al.* (2002) and Sotillo *et al.* (2001)
K5-Cdk4; p27 $^{-/-}$	Cooperation in IL tumors	100	3	Macias *et al.* (2008)
$Cdk4^{R24C/R24C}$; p27 $^{-/-}$	Strong cooperation and undifferentiated tumors	100	2	Sotillo *et al.* (2005)
$pRB^{+/-}$; $Pttg1^{-/-}$	IL tumors with decreased incidence versus pRB mutants	30	13	Chesnokova *et al.* (2005)
$pRB^{+/-}$; αGSU.PTTG1	Overexpression of securin cooperates in AP tumors	100	16	Donangelo *et al.* (2006)
Pituitary hypoplasia				
$Cdk4^{-/-}$	Defective proliferation and endocrine cell numbers	100	Postnatal	Rane *et al.* (1999)
$Securin^{-/-}$	Hypoplastic pituitary	ND	ND	Melmed (2003)

ND, Not determined.

2002), indicating the relevance of the pRB/E2F pathway in pituitary tumorigenesis. The sole overexpression of another E2F family member, E2f3, is not sufficient to produce pituitary tumors, although these transgenic mice develop pituitary hyperplasia (Lazzerini Denchi *et al.* 2005).

The genetic analysis of pRB in the mouse clearly demonstrated a tumor suppressor function for this protein, and specifically in endocrine organs such as the pituitary. By that time, pRB function in the cell cycle was not fully explored and the relationship with the pituitary was not obvious. More than 15 years later, the reasons for the special sensitivity of endocrine tissues and particularly the pituitary, to pRB lost are not understood yet. However, this close relationship is not restricted to pRB protein. In 1996, three groups reported multiple organ hyperplasia, including pituitary tumors in $p27^{Kip1}$ mutant mice (Fero *et al.* 1996, Kiyokawa *et al.* 1996, Nakayama *et al.* 1996). As in the pRB mutants, $p27^{Kip1}$-deficient mice developed pituitary tumors by the age of 12 months (Kiyokawa *et al.* 1996, Nakayama *et al.* 1996). Although, in both cases the animals developed IL tumors, they present differential patterns in both the histological phenotype and the gene profile expression (Chien *et al.* 2007). Soon after, a significant incidence of pituitary tumors was described in mice deficient in another cell cycle inhibitor, the member of the INK4 family $p18^{INK4c}$. Fifty percent of these animals developed aggressive pituitary tumors mostly from the IL by 15 months, although some tumors originated from the AP (Franklin *et al.* 1998). Deficiency in either of the other INK4 proteins, $p16^{INK4a}$, $p15^{INK4c}$,or $p19^{INK4d}$ does not result in pituitary tumors. However, genetic ablation of both $p16^{INK4a}$ and $p18^{INK4c}$ cooperates both in the incidence and the latency of the development of the pituitary tumors (median survival of 10 months; Ramsey *et al.* 2007). No cooperation in pituitary tumor suppression is observed between $p18^{INK4c}$ and $p15^{INK4b}$ (Latres *et al.* 2000) or $p19^{INK4d}$ (Zindy *et al.* 2001).

INK4 proteins specifically inhibit CDK4 and CDK6 kinases by competing with the obligate activator of these kinases, the cyclins. The relevance of INK4 proteins as key inhibitor of CDK4 and CDK6 is highlighted by a specific mutation in CDK4 (Arg24 to Cys) that prevents inhibition of this kinase by INK4 proteins. This mutation has been observed in both hereditary and spontaneous melanoma with low incidence (Malumbres & Barbacid 2001). When a Cdk4 R24C mutant protein is expressed in the mouse in substitution of the endogenous wild-type protein, these knock-in mice develop multiple tumors including frequent endocrine and mesenchymal tumors (Sotillo *et al.* 2001, Rane *et al.* 2002). Interestingly, pituitary tumors are also frequent (around 25% in all the studies) in these knock-in mice suggesting the relevance of CDK4 kinase activity in these neoplasias. Most of these pituitary tumors originated in the AP with an average latency of around 15 months.

One or several cell cycle pathways in pituitary tumorigenesis?

The former models suggest a clear relevance of the CDK (and their inhibitors INK4 or KIP)/pRB pathway in pituitary tumorigenesis. However, the results obtained from the combination of some of these mutations in the mouse suggest a more complex molecular network. The combined deletion of pRB and $p27^{Kip1}$ results in shorter latency of pituitary tumors in p27 (−/−); pRb (+/−) mice (Park *et al.* 1999). In addition, the expression of $p27^{Kip1}$ mRNA is reduced in pituitary tumors from pRb(+/−) mice, suggesting that $p27^{Kip1}$ downregulation is necessary for the tumorigenicity of the pituitary even in a pRb-null background. Similarly, although $p21^{Cip1}$-null mice do not develop pituitary tumors, this mutation cooperates with pRb mutation by decreasing the latency of pituitary tumors from 12 to 9 months (Brugarolas *et al.* 1998). Similarly, both $p27^{Kip1}$ and $p21^{Cip1}$ deficiency accelerates pituitary tumorigenesis in a $p18^{INK4c}$-null background (Franklin *et al.* 1998, 2000). This cooperation is dramatic in double $p27^{Kip1}$; $p18^{INK4c}$ mutants, which develop pituitary adenomas within 3 months (Franklin *et al.* 1998).

Since both INK4 and CIP/KIP proteins are CDK inhibitors, these results suggested that these molecules cooperate in tumor suppression by strongly inactivating CDK function in the pituitary (Fig. 3). INK4 proteins specifically inhibit CDK4/6 kinases, whereas CIP/KIP proteins seem to preferentially inhibit CDK2 and CDK1. In agreement with this model, no cooperation in pituitary tumor formation is observed in double Cdk4 R24C; p18-null mice (Sotillo *et al.* 2005). However, the introduction of the mutated Cdk4 R24C allele in a p27-null background dramatically accelerates the development of pituitary tumors that kill these mutant mice in 8–10 weeks (Sotillo *et al.* 2005). No cooperation in pituitary tumor development is observed in mice mutant for Cdk4 R24C and deficient in $p21^{Cip1}$ (Quereda *et al.* 2007). However, a dramatic cooperation in pituitary tumor development is observed in mutant mice carrying a combination of the Cdk4 R24C, p21-null, and P27-null alleles (V Quereda and M Malumbres, unpublished observations). These results, together with the cooperation observed between pRb and $p27^{Kip1}$ (Park *et al.* 1999), suggest the existence of two major pathways for G1/S phase deregulation in pituitary tumors. One branch is formed

of $p18^{INK4c}$/CDK4/pRB, whereas the other one is represented by $p27^{Kip1}$ and perhaps $p21^{Cip1}$ (Fig. 3).

The preference of CIP/KIP proteins for CDK family members other than CDK4/6 indicated that these inhibitors may target CDK2, the other interphase CDK involved in G1/S transition. However, $p27^{Kip1}$ deficiency provokes similar pituitary tumors in both Cdk2(+/+) and Cdk2(−/−) mice (Martin *et al.* 2005) indicating that CDK2 is dispensible for these tumors and it is therefore not the critical target of $p27^{Kip1}$. Whether the other major cell cycle protein, CDK1, is the critical target of $p21^{Cip1}$ or $p27^{Kip1}$ during pituitary tumor suppression has not been fully addressed yet.

The complexity in the molecular pathways involved in pituitary tumorigenesis has recently increased after a new mouse model that suggests possible oncogenic functions of $p27^{Kip1}$. In this model, the authors designed a $p27^{Kip1}$ mutant allele that does not bind cyclins and CDKs and is mostly localized to the cytoplasm (Besson *et al.* 2007). These knock-in mice developed more aggressive tumors than the $p27^{Kip1}$-null mice, and by 6 months all the animals showed aggressive pituitary tumors of the anterior lobe. This phenotype seems to be independent of the cell cycle inhibitory activity of $p27^{Kip1}$ and it may be related to the ability of $p27^{Kip1}$ to modulate stem cell function (Besson *et al.* 2007).

Finally, a completely new cell cycle pathway involved in pituitary oncogenesis is represented by PTTG1 (pituitary tumor transforming gene)/securin, an oncogenic molecule first identified in GH4 rat pituitary tumor cells (reviewed in Vlotides *et al.* (2007) and Salehi *et al.* (2008)). PTTG1 is involved in the mitotic checkpoint that prevents abnormal chromosome segregation (see below). In addition, this protein has multiple roles in cell cycle regulation at different stages (Fig. 4). The absence of this gene provokes a decrease in the incidence of pituitary tumors in pRB heterozygous mice, probably by triggering ARF/p53/p21-dependent senescence (Chesnokova *et al.* 2005, 2007). Overexpression of PTTG1 in the pituitary in transgenic mice provokes pituitary hyperplasia and focal microadenomas, and cooperates with pRB heterozygosity in higher incidence of tumors in the AP (Donangelo *et al.* 2006).

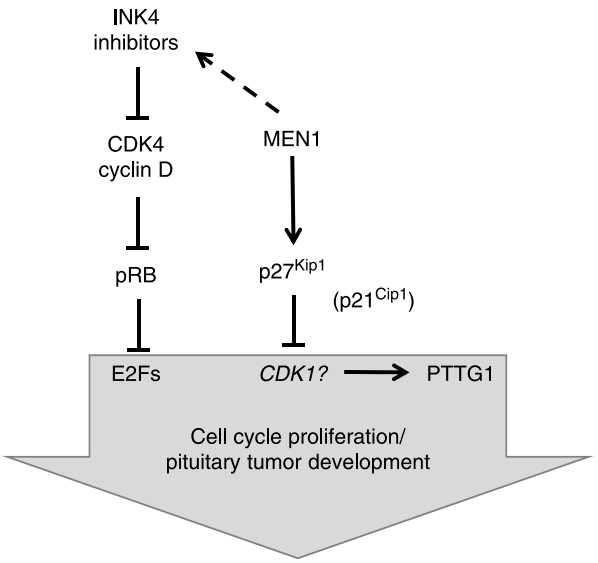

Figure 3 Major oncogenic and tumor suppressor pathways regulating the cell cycle in pituitary tumors.

Deregulation of the cell cycle in human pituitary disease

The experimental analysis of cell cycle control in mouse models predicts that several cell cycle mutations may be present in human pituitary diseases. Pituitary tumors are common intracranial neoplasms that cause significant morbidity through mass effects and/or the inappropriate secretion of pituitary hormones. Pituitary adenomas are common intracranial neoplasms, comprising 10–15% of diagnosed brain tumors (Landis *et al.* 1989). Data from autopsy studies suggest that pituitary adenomas develop in 17–25% of the population (Asa & Ezzat 2002, Ezzat *et al.* 2004). Approximately, 3·5–8·5% of all pituitary tumors are diagnosed prior to the age of 20 years (Keil and Stratakis 2008). About two-thirds of pituitary tumors express and secrete pituitary hormones and produce various endocrine syndromes. Overall, prolactinomas account for about 50% of pituitary adenomas. These adenomas cause hyperprolactinemia and subsequent problems associated to a high level of prolactin in blood (hypoestrogenism or amenorrhea in women or infertility in men). GH-producing adenomas are commonly associated with acromegaly and/or gigantism. ACTH-producing adenomas are associated with Cushing's or Nelson's syndromes (see below). TSH-producing tumors produce thyrotoxicosis, cardiac arrythmias, tremor, and weight loss. The rare gonadotroph adenomas and the major group of non-functionally or non-secreting adenomas result in hypogonadism, visual deficits, and headaches (Asa & Ezzat 2002, Melmed 2003, Ezzat & Asa 2006).

Several genetic and epigenetic alterations have been observed in pituitary tumorigenesis. Some classic oncogenes such as RAS or MYC are implicated in these endocrine tumors. H-RAS mutations (codon 12 (Gly→Val or Arg) or 18 (Ala→Tre)) have been reported only in pituitary carcinomas (Karga *et al.* 1992, Cai *et al.* 1994, Pei *et al.* 1994). c-MYC, on the other hand, is frequently overexpressed in all kind of pituitary tumors in a range between 20 and 50% depending on the type of the tumor (Woloschak *et al.* 1994, Wang *et al.* 1996). Among classic tumor-suppressor genes, p53

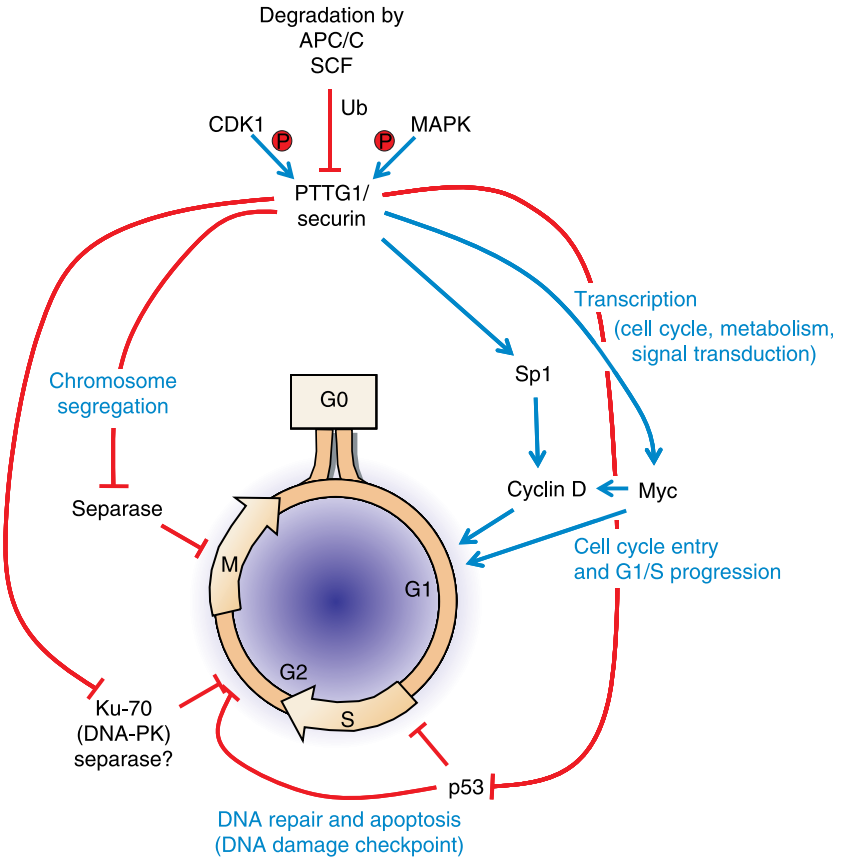

Figure 4 PTTG1/securin functions in the cell cycle (for a comprehensive review see Vlotides *et al.* (2007)).

accumulation (an indication of inactive p53 function) seems to be more relevant in Cushing's adenomas and invasive non-functional tumors than in non-functioning adenomas (Buckley *et al.* 1994, Thapar *et al.* 1996, Clayton *et al.* 1997). In addition to these classic cancer genes, a significant number of genetic or epigenetic alterations in pituitary tumors target several cell cycle regulators as described in the following paragraphs (Table 2). From these data, it has been estimated that more than 80% of pituitary tumors display alterations at least in one of the regulators of the G1/S transition of the cell cycle (Malumbres & Barbacid 2001).

Retinoblastoma protein

Although, early studies did not find loss of pRB alleles (Cryns *et al.* 1993, Zhu *et al.* 1994), later studies found loss of heterozygosity in the human pRB gene (*RB1*) in malignant or highly invasive pituitary tumors (Pei *et al.* 1995; Table 2). Several studies based on immunodetection in tumor sections found abnormal expression of pRB in different pituitary adenomas. In some cases, decreased expression correlates with hypermethylation of the pRB promoter (Simpson *et al.* 2000, Ogino *et al.* 2005) or deletion within the protein-pocket binding domain (Simpson *et al.* 2000).

Cyclins and cyclin-dependent kinase activity

Cyclin D1 and D3 are often overexpressed in pituitary tumors (Jordan *et al.* 2000, Turner *et al.* 2000, Saeger *et al.* 2001, Simpson *et al.* 2001*a*) with some evidence of cyclin D1 allelic imbalance in one fourth of the tumor samples analyzed (Hibberts *et al.* 1999). In general, although cyclin D1 is overexpressed in most pituitary tumor types, this overexpresion is more relevant in non-functional tumors. Cyclin E is also deregulated in human pituitary tumors, with a significant increase in corticotroph neoplasias from patients with Cushing's disease (Jordan *et al.* 2000). Despite the dramatic effect of Cdk4 hyperactivation in mouse models (Table 1), no CDK4 mutations have been identified in human pituitary tumors (Simpson *et al.* 2001*a*, Honda *et al.* 2003, Vax *et al.* 2003).

Table 2 Alteration in cell-cycle regulators in human pituitary tumors

Gene (symbol)	**Cancer-associated alteration** (incidence)	**Tumor type**	**References**
pRB (*RB1*)	LOH (100%)	Highly-invasive or malignant tumors	Pei *et al.* (1995)
	Promoter hypermethylation (60% of non-expressing pRB tumors)	Somatotrophinoma and non-secreting adenomas	Simpson *et al.* (2000)
	Promoter hypermethylation (35%)	Pituitary adenomas	Yoshino *et al.* (2007)
	Promoter hypermethylation (28·6%)	Pituitary adenomas	Ogino *et al.* (2005)
Cyclin D1 (*CCND1*)	Allelic imbalance (25%)	Invasive and non-invasive tumors	Hibberts *et al.* (1999)
	Overexpression (30–50%)	Somatotrophinomas & non-functioning tumors	Simpson *et al.* (2001*a*,*b*)
Cyclin D3 (*CCND3*)	Overexpression (68%)	Pituitary adenomas (all different types)	Saeger *et al.* (2001)
Cyclin E (*CCNE*)	Overexpression (37%)	Cushing's disease adenomas	Jordan *et al.* (2000)
Cyclin A (*CCNA1*)	Overexpression	Pituitary adenomas	Nakabayashi, *et al.* (2001)
$p16^{INK4a}$ (*CDKN2A*)	Promoter hypermethylation (90% of non-expressing p16 tumors)	Different pituitary tumors	Woloschak *et al.* (1997)
	Promoter hypermethylation (59%)	Pituitary adenomas	Yoshino *et al.* (2007)
	Promoter hypermethylation (71·4%)	Pituitary adenomas	Ogino *et al.* (2005)
	Reduced expression levels (62%)	Non-functioning adenomas or	Machiavelli, *et al.* (2008)
	Reduced expression levels (40%)	Macroadenomas (all different types)	Machiavelli *et al.* (2008)
$p15^{INK4b}$ (*CDKN2B*)	Promoter hypermethylation (32%)	Pituitary adenomas	Yoshino *et al.* (2007)
	Promoter hypermethylation (35,7%)	Pituitary adenomas	Ogino *et al.* (2005)
$p18^{INK4c}$ (*CDKN2C*)	Reduced expression levels	ACTH-secreting adenomas	Morris *et al.* (2005)
p27Kip1 (*CDKN1B*)	Reduced expression levels (75% less than 10% cells-expressing in the tumor)	Pituitary adenomas (all different types)	Bamberger *et al.* (1999)
	Reduced expression levels (100%)	Corticotropes & pituitary carcinomas	Lidhar *et al.* (1999)
JAB1 (*COPS5*)	Overexpression (100%)	Pituitary carcinomas	Korbonits *et al.* (2002)
$p21^{CIP1}$ (*CDKN1A*)	Reduced expression levels (71%)	Non-functioning adenomas	Neto, *et al.* (2005)
	Overexpression (77%)	Hormone-producing tumors	Neto *et al.* (2005)
	Overexpression (92%)	GH-producing tumors	Neto *et al.* (2005)
Securin (*PTTG1*)	Overexpression (90% pituitary tumors)	Pituitary adenomas (all different types)	Zhang *et al.* (1999)

INK4 inhibitors

Although point mutations in INK4 inhibitors are not frequent in human pituitary adenomas, the expression of $p16^{INK4a}$ and $p15^{INK4b}$ is often silenced. Silencing of the $p16^{INK4a}$ gene (*CDKN2A*) by hypermethylation was first reported in the late 90s (Woloschak *et al.* 1997). A detailed analysis suggested that *CDKN2A* methylation was confined to particular adenoma subtypes (Simpson *et al.* 1999) and these findings were subsequently confirmed by several other groups concluding that hypermethylation of the *CDKN2A* is the most common epigenetic deregulation in these neoplasias (Morris *et al.* 2005, Ogino *et al.* 2005, Yoshino *et al.* 2007). $p16^{INK4a}$ is able to inhibit cell proliferation in pituitary tumor cells in correlation with a shift in the phosphorylation status of pRB, suggesting the relevance of this CDK inhibitor in the activation of pRB and pituitary tumor suppression (Frost *et al.* 1999).

CIP/KIP inhibitors

Soon after the publication of the phenotype of $p27^{Kip1}$-deficient mice, several studies interrogated the alteration of this inhibitor in human tumors. Early studies detected no $p27^{Kip1}$ mutations in human pituitary tumors (Tanaka *et al.* 1997, Dahia *et al.* 1998). The fact that $p27^{Kip1}$ is haploinsufficient for tumor suppression (Fero *et al.* 1998), however, suggests that decreased expression may be relevant in tumor development. In fact, downregulation of $p27^{Kip1}$ protein expression is commonly observed in pituitary carcinomas and corticotroph adenomas, and recurrent human pituitary adenomas show lower $p27^{Kip1}$ protein levels than non-recurrent adenomas (Bamberger *et al.* 1999, Lidhar *et al.* 1999). $p27^{Kip1}$ mRNA levels are not generally decreased in tumors suggesting increased proteolysis of this cell cycle inhibitor in cancer (Bloom & Pagano 2003). Ubiquitin-mediated degradation of $p27^{Kip1}$ is controlled by SKP2, an F-box protein with diverse oncogenic functions (Frescas & Pagano 2008). Whether SKP2 is the relevant F-box protein for degradation of $p27^{Kip1}$ in pituitary tumors is not yet clear (Musat *et al.* 2002). Degradation of $p27^{Kip1}$ may also be induced by JAB1 (JUN activation domain-binding protein), a transcriptional cofactor for AP-1 (Chamovitz & Segal 2001). In addition to this function, JAB1 is able to translocate phosphorylated $p27^{Kip1}$ to

the cytoplasm for protein degradation by the proteasome. Some pituitary carcinomas display a small but significant increase in JAB1 levels possibly resulting in increased $p27^{Kip1}$ degradation (Korbonits *et al.* 2002). Although, genetic alterations in $p21^{Cip1}$ are not commonly observed, this inhibitor may also be down-regulated through epigenetic modifications in pituitary neoplasias (Yoshino *et al.* 2007, Zhu *et al.* 2008).

Although, the majority of pituitary tumors in humans are spontaneous, in some cases they are part of genetic syndromes predisposing to pituitary and other tumors. These inherited syndromes include multiple endocrine neoplasia (MEN)-1, carney complex, familial isolated pituitary adenomas, and the Cushing's and Nelson's syndromes (Melmed 2003, Beckers & Daly 2007, Keil & Stratakis 2008). The MEN-1 syndrome is characterized by predisposition to pituitary adenomas, parathyroid hyperplasia, and pancreatic endocrine tumors. Pituitary adenomas affect between 25 and 30% of MEN-1 patients (Burgess *et al.* 1998). These patients display germ line mutations in the *MEN1* gene, which increase the susceptibility to all major pituitary adenoma subtypes. MEN1 has been described as a direct regulator of $p27^{Kip1}$ and $p18^{INK4c}$ (Karnik *et al.* 2005, Milne *et al.* 2005), and loss of function of MEN1 results in down-regulation of these two inhibitors with the subsequent deregulation in cell proliferation. In recent mouse models, Men1 mutations cooperate with $p18^{INK4c}$ but not $p27^{Kip1}$ inactivation (Bai *et al.* 2007) suggesting that the MEN1 protein is mostly acting upstream of $p27^{Kip1}$ (Fig. 3). Recently, a mutation in *CDKN1B*, the rat gene encoding $p27^{Kip1}$, has been reported to be associated with a MEN-1-like syndrome in a murine model (Pellegata *et al.* 2006). A germ line nonsense mutation in the human *CDKN1B* gene was also identified in a *MEN1* mutation-negative patient presenting with pituitary and parathyroid tumors. Expanded pedigree analysis showed that the $p27^{Kip1}$ mutation was associated with the development of an MEN-1-like phenotype in multiple generations (Pellegata *et al.* 2006).

PTTG1/securin

PTTG1 was initially identified through a differential display analysis of gene expression in rat pituitary tumor cells (Pei & Melmed 1997). PTTG1, also known as securin, is an inactivating partner of separase, the major effector for chromosome segregation during mitosis (Zou *et al.* 1999). PTTG1 is overexpressed in more than 90% of all type of pituitary tumors (Zhang *et al.* 1999). In addition, this protein is frequently overexpressed in metastatic or genomically instable tumors, suggesting a relevant role for securin in tumor progression (Perez de Castro *et al.* 2007). Securin is regulated by CDK1-mediated phosphorylation (Holt *et al.* 2008) suggesting a link between the control of the cell cycle by CDKs and PTTG1 function (Fig. 3). Despite the frequent deregulation of PTTG1 in pituitary and other tumors, it is not clear yet whether its oncogenic role is mediated by its mitotic functions or the ability of PTTG1 or modulate DNA repair or Sp1-mediated transcription (Vlotides *et al.* 2007; Fig. 4).

Future perspectives and therapeutic implications in pituitary disease

The implication of cell cycle deregulation in pituitary tumorigenesis is well established from experimental data in mouse models (Table 1) and the molecular pathology of human tumors (Table 2). Most cell cycle mutations affect regulators of the G1/S transition in the cell cycle, including the CDK4/pRB pathway and cell cycle inhibitors such as $p27^{Kip1}$ (Malumbres & Barbacid 2001). The role of the pioneer pituitary tumor oncogene PTTG1 is not clear at present, although it may participate in tumor development at different levels. Overall, these mutations provoke a hyperactive cell cycle that ensures unscheduled proliferation and genomic instability in pituitary tumors.

On the other hand, defective cell cycle function also affects pituitary homeostasis. Cdk4 deficient mice are smaller than wild-type littermates and display partial sterility (Rane *et al.* 1999). These phenotypes are linked to hypomorphic pituitaries with a significant decrease in hormone-producing cells. In particular, Cdk4 is required for post-natal proliferation of somato/lactotrophs of the pituitary (Moons *et al.* 2002, Jirawatnotai *et al.* 2004). Some recent results suggest that Cdk4 may also modulate cell proliferation in specific pituitary progenitor cells (Macias *et al.* 2008). Re-expression of Cdk4 in the pituitary rescues the sterility indicating that this defect is secondary to the defects in hormone-expressing cells in the pituitary (Martin *et al.* 2003). However, that re-expression of Cdk4 in the pituitary does not rescue the smaller size of Cdk4-null mice suggesting that dwarfism in these animals is not due to pituitary dysfunction (Martin *et al.* 2003).

Pttg1-deficient mice also display pituitary hypoplasia and decreased proliferation of pancreatic β-cells (Melmed 2003, Vlotides *et al.* 2007). The similarity between Cdk4 and Pttg1 deficiency is striking, although the molecular reasons are unclear. To what extent these cell cycle control pathways contribute to pituitary development and homeostasis is not fully understood yet. However, these experimental results may suggest a relevant relationship between cell cycle regulators and the ability of the pituitary to develop and to respond to physiological stresses. Given the relevance of cell cycle regulators in the correct function of stem cells (Janzen *et al.* 2006, Jablonska *et al.* 2007, Pei *et al.* 2007, Macias

et al. 2008), it is tempting to speculate on the relevance of the cell cycle in pituitary stem cell self-renewal and its implications in pituitary syndromes and tumors.

The observed deregulation of the cell cycle in pituitary disease has important consequences in the treatment of these pathologies. Current treatments in pituitary tumors target neuroendocrine receptors to block hormone–receptor signaling through different pathways (Heaney & Melmed 2004). Currently used drugs include dopamine-receptor agonists and somatostatin analogues. These substances are used to suppress excess hormone secretion and proliferation of pituitary cells, although they also produce several side effects (Heaney & Melmed 2004). The frequent overexpression of cyclins and inactivation of cell cycle inhibitors such as INK4 proteins suggests that CDK hyperactivation is a common theme in pituitary neoplasias. Several small molecular CDK inhibitors are now being evaluated for cancer therapy in many different tumor types (Malumbres *et al.* 2008, Perez de Castro *et al.* 2008). Although, these drugs have not been clinically tested in pituitary tumors, pre-clinical studies suggest that CDK inhibitors may be effective for treating pituitary diseases, at least in individuals with cell cycle mutations that specifically affect this pathway (Sotillo *et al.* 2005). A better knowledge of the specific genetic and epigenetic alterations in human patients will be necessary to select the right combination of current treatments or to propose new therapeutic approaches. Current and future genetic models in the mouse will help us to understand the development of pituitary disorders and to evaluate these therapies before their use in the Clinic.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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