REVIEW

Role of the high mobility group A proteins in the regulation of pituitary cell cycle

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Abstract

Pituitary cells are particularly sensitive to alterations of the cell cycle machinery. In fact, mutations affecting expression of proteins critical for cell cycle progression, including retinoblastoma protein, cyclins D1 and D3, p16^{INK4A}, and p27^{kip1}, are frequent in human pituitary adenomas. Similarly, both targeted disruption and overexpression of either cell cycle inhibitors or activators, respectively, lead to the development of pituitary adenomas in mice. Recent evidence has added the high mobility group A (HMGA) proteins as a new class of cell cycle regulators that play significant roles in the pathways that lead to pituitary tumor evolution in both humans and experimental animal models. Here, we first review the role of the cell cycle in pituitary tumorigenesis, as witnessed by human pathology and transgenic mice; and then, we focus on HMGA proteins and their cell cycle-related role in pituitary tumorigenesis.

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Introduction

The pituitary gland is a critical endocrine organ on which growth, metabolism, reproduction, and homeostasis depend. In mammals, it consists of three lobes: the anterior and the intermediate lobes form the adenohypophysis, while the posterior lobe represents the neurohypophysis. The adenohypophysis secretes seven different hormones: GH, TSH, prolactin (PRL), FSH, LH, ACTH, and melanocyte-stimulating hormone, whereas the neurohypophysis releases two hypothalamic neuropeptides, vasopressin and oxytocin. In the adenohypophysis, hormones are produced by different cell types, whose absolute and relative number may vary during adult life depending on the endocrine demands (Sasaki 1988, Levy 2002). The regulation of the proliferative ability of pituitary cells in adulthood is not well established, although the presence of a side population of stem/progenitor cells, which may contribute to cell renewal in the adult pituitary, has been described (Chen *et al.* 2005, 2009). Recent experimental data suggest that cell cycle regulators may have significant implications in the biology of putative progenitor cells and pituitary homeostasis (Janzen *et al.* 2006, Macias *et al.* 2008). Indeed, cell

cycle plays a key role in the development of pituitary adenomas as assessed in humans and mice.

The cell cycle

The cell cycle is the process by which cells divide into daughter cells. It comprises a defined sequence of events, which include: synthesis of DNA (S phase), mitosis (M phase), and two phases of growing and preparation for following steps (G₁ and G₂ phases; Fig. 1). A reversible quiescence phase (G₀), in which cells 'take a rest' in the absence of mitogenic signals, is also considered part of the cell cycle (Norbury & Nurse 1992). At particular stages of the cell cycle, cells encounter a restriction point and four checkpoints that enable the correct progress and completion of each cycle. The restriction point (R) is a specific molecular event, which is set between early and late G₁ phases, after which cells can proliferate independently from mitogenic stimuli (Pardee 1974), whereas checkpoints are surveillance mechanisms and quality controls of the genome to maintain genomic integrity, which are set between G₁ and S, intra-S, between G₂ and M and intra-M (Hartwell & Weinert 1989).

Figure 1 Schematic representation of the main cell cycle regulating pathways affected in pituitary tumors. The blue arrow indicates the restriction point. A, B, D, and E define the different cyclins. Stimulatory or inhibitory effects are indicated by an arrow or a bar, respectively, at the end of a line. Dotted ovals indicate group proteins and protein complexes that share similar functions and regulations.

Both activating and inhibitory signals, represented by cyclins coupled to cyclin-dependent kinases (CDKs) and CDK inhibitors (CKI), respectively, finely regulate the progress of the cell through the different phases of the cell cycle (Morgan 1997, Vidal & Koff 2000).

Among the activators, D-type cyclins, coupled to CDK4 and CDK6, promote the progression through G₁; E-type cyclins, coupled to CDK2, are involved in the G₁/S transition; A-type cyclins, coupled to CDK2 and CDK1, participate in the S/G₂ transition and progression through G₂ respectively; B-type cyclins, coupled to CDK1, are involved in the G₂/M transition; cyclins A and B also complex with CDK1/CDC2/p34 to form the mitosis-promoting factor whose activity peaks at G₂/M transition, being required for the cell to enter M phase (Pines & Hunter 1989).

The transcription factor family E2F plays a critical role in G₀/G₁, R, and G₁/S transitions, by allowing transcription of D- and E-type cyclins. Consistently, the gatekeeper retinoblastoma protein (pRB) negatively regulates G₁ entering, progression, and transition to S by blocking the activity of factors responsible for cell cycle progression, which include the E2Fs. CDK4, CDK6, and CDK2, complexed to D- and E-type cyclins, mediate phosphorylation of pRB, thus provoking the release and activation of E2F factors from pRB (Fig. 1). The pRB/E2Fs complex can also be

regulated by acetylation/deacetylation, mediated by histone acetylases and deacetylases (Magnaghi-Jaulin *et al.* 1998, Martínez-Bálabas *et al.* 2000).

The action of the cyclin-CDK complexes is counteracted by specific CKIs either by blocking the action of CDKs or by impairing substrate/ATP access. The CKIs belong to two families: the INK4 and the Cip/Kip families. The INK4 family consists of four members (p16^{INK4a}, p15^{INK4b}, p18^{INK4c}, and p19^{INK4d}), which inhibit the G₁/S transition by blocking CDK4 and CDK6. Conversely, the Cip/Kip family, which includes p21^{Cip1}, p27^{Kip1}, and p57^{Kip2}, has different roles depending on the cyclin-CDK complexes, which they bind to. Association with cyclin-CDK2 and cyclin-CDK1 blocks their activity, whereas binding to cyclin-CDK4 and cyclin-CDK6 can upregulate their activity (Cheng *et al.* 1999, Sherr & Roberts 1999; Fig. 1).

Negative regulation of the cell cycle is not restricted to CKIs. One critical target of negative regulation that acts in M phase is a protein named securin or PTTG1 (Pei & Melmed 1997). During metaphase, replicated paired sister chromatids are held together by the cohesin complex, which is degraded by the proteolytic protein separase, during the metaphase to anaphase transition, to allow the separation of sister chromatids to proceed to diploid daughter cells (Uhlmann *et al.* 1999). The activity of separase is inhibited by the

interaction with securin, whose proteosomal degradation by the ubiquitin ligase APC is crucial for the completion of the anaphase process (Zou *et al.* 1999). In addition to this securin function, recent reports indicate a role for PTTG1 in modulation of the G₁/S phase transition by interacting with Sp1 and regulating the transcriptional activity on the cyclin D3 promoter (Tong *et al.* 2007). Interestingly, securin/PTTG1 is regulated by CDK1-mediated phosphorylation (Holt *et al.* 2008) suggesting a link between the control of the cell cycle by CDKs and securin function.

Cell cycle dysregulation in human pituitary tumorigenesis

Cell cycle dysregulation is the main pathogenetic event in the development of pituitary tumors. In fact, it has been estimated that more than 80% of human pituitary tumors display alterations at least in one of the regulators of the G₁/S transition of the cell cycle (Malumbres & Barbacid 2001; Table 1). These alterations are frequently represented by epigenetic events that target several cell cycle regulators, leading to overexpression of cyclins (mainly D1, D3, E, B1, and B2), as well as to downregulation of CKIs (mainly p16^{INK4A}, p15^{INK4B}, p27^{Kip1}, and p21^{Cip1}) and pRB expression (Farrell & Clayton 2003). Cyclins D1 and D3, as well as cyclin E, are frequently overexpressed in all subtypes of human pituitary adenomas, with prevalence in nonfunctional pituitary adenomas (NPPA) for the D-type (Jordan *et al.* 2000, Turner *et al.* 2000, Saeger *et al.* 2001, Simpson *et al.* 2001) and in corticotroph

tumors for the E-type cyclins (Jordan *et al.* 2000). A cyclin D1 gene allelic imbalance has also been described in about 25% of analyzed adenomas (Hibberts *et al.* 1999). B-type cyclins have recently been described as overexpressed in many human pituitary adenomas, with prevalence in prolactinomas (Wierinckx *et al.* 2007, De Martino *et al.* 2009b). Silencing of the gene encoding p16^{INK4A} by methylation is frequent in pituitary, mainly NPPA (Woloschak *et al.* 1997, Simpson *et al.* 1999a, Morris *et al.* 2005, Ogino *et al.* 2005, Yoshino *et al.* 2007). Downregulation of p27^{Kip1} protein expression, likely due to a JAB1-mediated increased proteolysis (Korbonits *et al.* 2002), is frequent in pituitary carcinomas and ACTH-secreting adenomas (Bamberger *et al.* 1999, Lidhar *et al.* 1999). Conversely, downregulation of p21^{Cip1} in pituitary adenomas may be due to epigenetic modifications (Yoshino *et al.* 2007, Zhu *et al.* 2008). Similarly, frequent loss of pRB expression observed in 27% of somatotrophinomas (Simpson *et al.* 1999b) was mainly due to methylation and/or microdeletion of the RB1 gene (Simpson *et al.* 2000).

In addition to these classical cell cycle-related proteins, the overexpression of PTTG1 has been observed in more than 90% of all types of pituitary tumors (Zhang *et al.* 1999). Finally, as described in more detail in a subsequent section of this review, overexpression of high mobility group A (HMGA) proteins has been shown to play a critical role in development of pituitary adenomas, mainly prolactinomas and GH/PRL-secreting adenomas, by mechanisms involving dysregulation of cell cycle-related proteins (Fedele *et al.* 2002, 2006, Finelli *et al.* 2002, De Martino *et al.* 2009b).

Table 1 Major cell cycle-related proteins altered in human pituitary tumors

	Cell cycle transition	Pituitary tumor subtype	Incidence (%)	References
Protein				
Cyclin D1	G ₁ /S	GH secreting	30	Simpson <i>et al.</i> (2001)
		Nonfunctioning	50	Simpson <i>et al.</i> (2001)
Cyclin D3	G ₁ /S	All different types	68	Saeger <i>et al.</i> (2001)
Cyclin E	G ₁ /S	ACTH secreting	37	Jordan <i>et al.</i> (2000)
Cyclin B1	G ₂ /M	Invasive PRL	89	Wierinckx <i>et al.</i> (2007)
		Aggressive PRL	100	Wierinckx <i>et al.</i> (2007)
Cyclin B2	G ₂ /M	All different types	100	De Martino <i>et al.</i> (2009b)
p16 ^{INK4a}	G ₁ /S	Nonfunctioning	70	Simpson <i>et al.</i> (1999a)
		Nonfunctioning	62	Machiavelli <i>et al.</i> (2008)
		All different types	40	Machiavelli <i>et al.</i> (2008)
p27 ^{Kip1}	G ₁ /S	ACTH secreting	100	Lidhar <i>et al.</i> (1999)
		Carcinomas	100	Lidhar <i>et al.</i> (1999)
JAB1	G ₁ /S	Carcinomas	100	Korbonits <i>et al.</i> (2002)
p21 ^{Cip1}	G ₁ /S	Nonfunctioning	71	Neto <i>et al.</i> (2005)
		Hormone secreting	77	Neto <i>et al.</i> (2005)
		GH secreting	92	Neto <i>et al.</i> (2005)
pRB	G ₁ /S	GH secreting	27	Simpson <i>et al.</i> (1999b)
		All different types	35	Yoshino <i>et al.</i> (2007)
Securin	G ₁ /S and G ₂ /M	All different types	90	Zhang <i>et al.</i> (1999)
HMGA1/2	G ₁ /S and G ₂ /M	All different types	100	De Martino <i>et al.</i> (2009b)

Cell cycle-related mouse models of pituitary adenomas

In keeping with the critical role of cell cycle dysregulation in pituitary tumorigenesis, targeted disruption or overexpression of cell cycle regulators frequently causes onset of pituitary adenomas (Table 2). The first mouse model of cell cycle-related pituitary tumorigenesis came from the phenotype of knockout mice for pRb (Jacks *et al.* 1992). The heterozygous animals developed pituitary tumors of the intermediate lobe (IL) arising from cells in which the wild-type Rb allele was absent. Loss of either E2F1 or E2F4 reduced pituitary tumorigenesis in these mice, suggesting that loss of pRB induces the onset of pituitary tumors by activating E2F factors (Yamasaki *et al.* 1998, Lee *et al.* 2002). Consistently, deregulated E2F activity in E2f3-transgenic mice induces hyperplasia of the pituitary gland (Lazzerini Denchi *et al.* 2005). Subsequently, three different groups reported the characterization of another mouse model of pituitary tumorigenesis: the knockout mice for p27^{kip1} (Fero *et al.* 1996, Kiyokawa *et al.* 1996, Nakayama *et al.* 1996). As in the pRb mutants, p27^{kip1}-null mice develop pituitary adenomas involving IL melanotrophs, even though differences in terms of gene expression and tumor phenotypes have been observed between the two models (Chien *et al.* 2007).

Therefore, despite the fact that deletions of p27^{kip1} or Rb cause IL tumors in murine models, they may have different mechanisms of tumor suppression. Indeed, as already described above, in addition to cyclin D complexes, which act upstream of pRB, p27^{kip1} inhibits both Cdk1 and Cdk2, both of which are capable of phosphorylating substrates other than pRB (Fig. 1). Crossing of pRb^{+/-} and p27^{-/-} mice lead to double mutants having a significantly reduced tumor latency period compared with single mutants (Park *et al.* 1999). Intriguingly, a recent new mouse model of pituitary tumorigenesis, the knock-in mice depleted of the Cdk inhibitory function of p27, develops pituitary adenomas larger and more vascular, causing more damage to surrounding tissue than those developing in p27-null mice (Besson *et al.* 2007). Interestingly, a recent study highlighted the importance of the mouse strain on pituitary tumor evolution, since it showed that 129Sv animals are inherently predisposed to IL pituitary tumors and that Rb^{+/-} mice in a C57BL/6 background develop high-penetrance anterior pituitary (AP) tumors (Leung *et al.* 2004). Another Cdk inhibitor, p18^{ink4c}, has been shown to be crucial in the homeostasis of the pituitary cell cycle. In fact, p18^{-/-} mice develop pituitary adenomas in both intermediate and anterior lobes (Franklin *et al.* 1998). Interestingly, crossing of p18-null mice with mice

Table 2 Mouse models of pituitary tumorigenesis caused by cell cycle dysregulation

	Pituitary phenotype	Incidence (%)	Latency (months)	References
Mice				
pRb ^{+/-}	IL adenomas	100	16	Jacks <i>et al.</i> (1992)
		100	11	Brugarolas <i>et al.</i> (1998)
		100	8	Lazzerini Denchi <i>et al.</i> (2005)
		100	9	Leung <i>et al.</i> (2004)
(129/sv background)	IL adenomas	100	9	Leung <i>et al.</i> (2004)
(C57BL/6 background)	AP adenomas	70	12	Leung <i>et al.</i> (2004)
ER-E2f3 ^{TG}	Hyperplasias	100	3	Lazzerini Denchi <i>et al.</i> (2005)
p27 ^{-/-}	IL adenomas	100	7	Kiyokawa <i>et al.</i> (1996)
		100	2	Fero <i>et al.</i> (1996)
		50	3	Nakayama <i>et al.</i> (1996)
		90	7	Park <i>et al.</i> (1999)
pRb ^{+/-} ; p27 ^{-/-}	IL adenomas	100	9	Brugarolas <i>et al.</i> (1998)
pRb ^{+/-} ; p21 ^{-/-}	IL adenomas	100	6	Besson <i>et al.</i> (2007)
p27 ^{CK-CK}	Aggress. AP adenomas	100	6	Besson <i>et al.</i> (2007)
p18 ^{-/-}	IL and AP adenomas	50	15	Franklin <i>et al.</i> (2000)
p18 ^{-/-} ; p16 ^{-/-}	IL and AP adenomas		10	Ramsey <i>et al.</i> (2007)
p18 ^{-/-} ; p27 ^{-/-}	IL and AP adenomas	100	3	Franklin <i>et al.</i> (1998)
p18 ^{-/-} ; p21 ^{-/-}	IL and AP adenomas	90	13	Franklin <i>et al.</i> (2000)
Cdk4 ^{R24C/R24C}	AP adenomas	25	15	Rane <i>et al.</i> (2002)
		22	15	Sotillo <i>et al.</i> (2001)
		100	2	Sotillo <i>et al.</i> (2005)
		100	4	Donangelo <i>et al.</i> (2006)
Cdk4 ^{R24C/R24C} ; p27 ^{-/-}	Undiff. adenomas	100	2	Donangelo <i>et al.</i> (2006)
Pttg1 ^{TG}	Hyperplasias	100	4	Donangelo <i>et al.</i> (2006)
Pttg1 ^{TG} ; pRb ^{+/-}	IL and AP adenomas	100	11	Fedele <i>et al.</i> (2002)
Hmga2 ^{TG}	AP adenomas	85 (females)	6 (females)	Fedele <i>et al.</i> (2002)
		40 (males)	18 (males)	Fedele <i>et al.</i> (2002)
Hmga2/T ^{TG}	AP adenomas	100 (females)	6 (females)	Fedele M (unpublished data)
Hmga1 ^{TG}	AP adenomas	80 (females)	12 (females)	Fedele <i>et al.</i> (2005)
		15 (males)	16 (males)	Fedele <i>et al.</i> (2005)

deficient for other Cdk inhibitors, such as p16, p27, or p21, led to double mutant mice with increased incidence and decreased latency in the development of pituitary adenomas as compared with single mutant mice (Franklin *et al.* 1998, 2000, Ramsey *et al.* 2007). Therefore, the existence of two major pathways for G₁/S phase dysregulation in pituitary tumors has been suggested: one involving p18/CDK4/pRB, and the other one involving p27 and p21 (Quereda & Malumbres 2009). Consistently, knock-in mice, which express a Cdk4 mutant originally found in human melanoma (Wölfel *et al.* 1995) and which are insensitive to INK4 inhibitors (Cdk4 R24C), develop several tumors, including pituitary adenomas (Sotillo *et al.* 2001, Rane *et al.* 2002). Crossing of Cdk4^{R24C/R24C} mice with mice deficient for p27 (but not with those deficient for p18) generates mice that develop pituitary adenomas with higher incidence and shorter latency than compound Cdk4 knock-in mice (Sotillo *et al.* 2005). Moreover, a dramatic cooperation in pituitary tumor development has been observed in mutant mice carrying a combination of the Cdk4 R24C, p21-null, and p27-null alleles (Quereda & Malumbres 2009).

Another protein involved in cell cycle regulation at different stages, and critical in pituitary alterations in mice, is PTTG1/securin. Indeed, transgenic mice overexpressing Pttg1 in the pituitary develop pituitary hyperplasia and focal microadenomas. Moreover, their crossing with pRb^{+/-} mice led to the development of AP adenomas (Donangelo *et al.* 2006), whereas Pttg1 deletion results in pituitary p21 induction and abrogates tumor development in Rb^{+/-} mice (Chesnokova *et al.* 2008).

Finally, mice overexpressing either members of the Hmga family (Hmga1 and Hmga2), or the truncated mutant Hmga2 (Battista *et al.* 1999), develop large and hypervascular AP adenomas (Fedele *et al.* 2002, 2005). These proteins play key roles in a wide range of biological processes including regulation of cell cycle (see below).

The HMGA proteins

The HMGA protein family includes HMGA1a and HMGA1b, which are encoded by the *HMGA1* gene through an alternative splicing (Johnson *et al.* 1989), and the closely related HMGA2 protein (Manfioletti *et al.* 1991).

The HMGA1a, HMGA1b, and HMGA2 proteins are composed of 107, 96, and 108 amino acid residues respectively. Each of them contains three basic domains, named AT-hooks, through which they bind DNA in AT-rich regions inside the minor groove (Elton *et al.* 1987, Reeves & Nissen 1990), and an acidic carboxy-terminal region, which contains several hydroxylic amino acids that are phosphorylated by casein kinase II. These proteins play key roles in chromatin architecture and

gene expression control by serving as generalized chromatin modifiers, either enhancing or suppressing the ability of several transcriptional factors to act on transcriptional regulation (Thanos & Maniatis 1995).

Both *HMGA* genes are widely expressed during embryogenesis, whereas they are absent or low expressed in adult cells and tissues (Zhou *et al.* 1995, Chiappetta *et al.* 1996, Rommel *et al.* 1997, Anand & Chada 2000). Recently, it has been shown that HMGA2 is highly expressed in neural fetal and young adult stem cells, being required for its self-renewal, relative to old adult stem cells, by negatively regulating *p16^{In4a}* and *p19^{Arf}* expression in fetal and young adult, but not in old adult, stages (Nishino *et al.* 2008).

HMGA overexpression represents a common feature of malignant neoplasias and a poor prognostic index since it often correlates with the presence of metastases and with a reduced survival (Fusco & Fedele 2007). The *HMGA* genes have a critical role also in the development of benign tumors of mesenchymal origin including lipomas. In fact, rearrangements of the *HMGA2* gene, due to 12q13–15 chromosomal translocations, have been frequently detected in these tumors (Ashar *et al.* 1995, Schoenmakers *et al.* 1995). In most of these cases, the HMGA2 modifications comprise truncation of the gene with the loss of the sequences coding for the C-tail and the 3'-UTR regulatory region that contains multiple target sites for *let-7*, one of the founding members of the microRNA (miR) family (Lee & Dutta 2007, Mayr *et al.* 2007). Therefore, this truncation would result in HMGA2 overexpression due to the lack of the inhibitory effect by *let-7*.

As far as HMGA1 is concerned, chromosomal breakpoints causing rearrangements have not been found intragenically but are found either upstream or downstream of the *HMGA1* gene, and sometimes within an 80 kb surrounding region (Kazmierczak *et al.* 1996, 1998).

Different studies, both *in vitro* and *in vivo*, have demonstrated that overexpression of the HMGA proteins is a necessary event in cell transformation: a) the block of HMGA1 protein synthesis by an antisense methodology prevents rat thyroid malignant cell transformation by acute murine retroviruses (Berlingieri *et al.* 1995, 2002); b) a recombinant adenovirus carrying *HMGA1* sequences in an antisense orientation, and able to suppress HMGA1 protein synthesis, induces cell death in two human thyroid anaplastic carcinoma cell lines, but not in normal thyroid cells (Scala *et al.* 2000); c) increased expression of both HMGA1 and HMGA2 proteins leads to neoplastic transformation of Rat1a fibroblasts with anchorage-independent cell growth (Wood *et al.* 2000); d) shRNA-mediated HMGA1 silencing on lung and pancreatic carcinomas resulted in significant reductions in anchorage-independent proliferation in

soft agar (Liau *et al.* 2007); and e) transgenic mice overexpressing either the *Hmga1* or *Hmga2* gene, under the transcriptional control of different promoters, develop several neoplasias including abdominal/pelvic lipomatosis, lipomas, mixed GH/PRL-secreting pituitary adenomas (Battista *et al.* 1999, Arlotta *et al.* 2000, Fedele *et al.* 2002), fibroadenomas of the breast, salivary gland adenomas (Zaidi *et al.* 2006), and mature T-cell lymphomas (Baldassarre *et al.* 2001, Xu *et al.* 2004).

HMGA proteins in pituitary tumorigenesis

Several pieces of evidence support a critical role of HMGA2 overexpression in the development of pituitary adenomas, particularly in prolactinomas that represent the most frequent histotype. Indeed, by dual-color interphase fluorescence *in situ* hybridization analysis using HMGA2-specific PACs and BACs, it was found that the *HMGA2* locus was amplified in seven of the eight prolactinoma samples examined. The cytogenetic manifestations of elevated HMGA2 concentrations ranged from simple trisomy to tetrasomy of 12 and der(12) chromosomes to marker chromosomes bearing 12q14–15-derived regions (where the *HMGA2* gene is located). Reverse transcription PCR, western blot, and immunohistochemical analysis showed HMGA2 overexpression in prolactinomas bearing rearrangement of regions 12q14–15 (Finelli *et al.* 2002). Conversely, the role of HMGA2 in development of NFPA is less evident since even though HMGA2 overexpression was observed in 12 out of 18 samples analyzed, the upregulation of the gene could be associated with amplification and/or rearrangement of the *HMGA2* locus only in two cases (Pierantoni *et al.* 2005). Recently, HMGA2 expression has been analyzed using immunohistochemistry with respect to various clinicopathologic factors in 98 pituitary adenomas (Qian *et al.* 2009). Overexpression of HMGA2 was observed in 39% of pituitary adenomas compared with normal adeno-hypophyseal tissue, and it was frequently found in PRL-, ACTH-, or FSH/LH-secreting adenomas and in NFPA, but relatively rare in GH- and mixed GH/PRL-secreting adenomas. HMGA2 expression was significantly associated with tumor invasion and was significantly higher in grade IV than in grades I, II, and III adenomas (Qian *et al.* 2009).

The amplification of the *HMGA2* locus mainly accounts for HMGA2 overexpression at least in prolactinomas. However, it is likely that increased dosage of chromosome 12 is insufficient to drive up activation of the *HMGA2* gene, since it was found in NFPA either expressing or not expressing the *HMGA2* gene. Recent studies support the idea that decreased expression of miRs, able to modulate HMGA protein

levels, may partially account for the abundant HMGA2 protein levels in pituitary adenomas. Indeed, decreased expression of let-7 was observed in 23 of 55 (42%) adenomas with an inverse correlation between let-7 and HMGA2 expressions (Qian *et al.* 2009). Moreover, it has been shown that miRs 15, 16, and 196, which target the *HMGA* mRNAs (De Martino *et al.* 2009a, Kaddar *et al.* 2009, Palmieri *et al.* manuscript in preparation), are downregulated in human prolactinomas (Bottoni *et al.* 2005, Palmieri *et al.* manuscript in preparation).

The critical role of HMGA2 protein in pituitary oncogenesis is validated by the phenotype of transgenic mice overexpressing a truncated or a wild-type *Hmga2* gene under the transcriptional control of the strong and ubiquitous CMV promoter (Fedele *et al.* 2002). In fact, most of these mice (85% of females and 40% of males) showed the onset of pituitary adenomas secreting PRL and GH (Fedele *et al.* 2002).

Differently from *HMGA2*, the causal role of *HMGA1* gene in pituitary carcinogenesis is still to be defined. In fact, even though both *HMGA1* and *HMGA2* transcripts have been found upregulated in a panel of 45 human pituitary adenomas (De Martino *et al.* 2009b), and transgenic mice overexpressing *Hmga1* develop pituitary adenomas secreting PRL and GH (Fedele *et al.* 2005), no rearrangements of the *HMGA1* locus have been found. Moreover, the detection of HMGA1 overexpression in all pituitary adenomas subtypes seems to indicate that HMGA1 induction may represent a secondary event in the tumorigenesis of pituitary gland.

HMGA2 and the pituitary cell cycle

Recent pieces of evidence indicate that HMGA2 protein is involved in the development of pituitary adenomas by interfering with the cell cycle machinery. Indeed, it has been demonstrated in the mouse model of *Hmga2* transgenic mice that the positive role of HMGA2 in pituitary cell proliferation is due to the interaction with the RB-E2F pathway, suggesting a new class of cell cycle-related proteins: the suppressors of the cell cycle inhibitors (Fedele *et al.* 2006). In more detail, HMGA2 binds to pRB A/B pocket domain thereby displacing the HDAC1 protein from pRB. This displacement results in the recruitment of HDAC to the E2F1 target promoters and acetylation of both histones and other proteins including E2F1. The acetylation of histones opens up the chromatin and facilitates gene transcription. Moreover, acetylation of E2F1 augments its DNA binding and stabilizes the protein in its free active form, thereby enhancing the expression of the E2F target genes, such as *CDC6* and *TK1* that promote the G₁/S transition. The crucial role of enhanced E2F1 activity in

the onset and progression of pituitary adenomas was validated by the rescue of the adenomatous phenotype when transgenic mice overexpressing the *Hmga2* gene were mated with *E2f1* knockout mice. In fact, the majority of double mutant mice did not develop tumors, and only a few of them showed small and slow growing adenomas (Fedele *et al.* 2006).

The alteration of cell cycle as a critical event in the induction of pituitary adenomas by the *HMGA2* gene seems further confirmed by the direct role of HMGA proteins in the upregulation of cyclin B2 gene transcription, leading to overexpression of cyclin B2 in pituitary adenomas developed by mice carrying *Hmga* transgenes (De Martino *et al.* 2009b). Consistently, an increased expression of *CCNB2*, the human gene coding for the cyclin B2 protein, directly correlates with *HMGA1* and *HMGA2* expression in human pituitary adenomas of different histotypes (De Martino *et al.* 2009b). Since cyclin B2, complexed with CDK1, plays a critical role in regulating the G₂/M phase transition of the cell cycle, it is reasonable to hypothesize that cyclin B2 induction by HMGA proteins may contribute to increase cell proliferation of the pituitary cells where HMGA proteins are overexpressed. Interestingly, the analysis of the mRNA expression profile of the pituitary adenomas arising in *Hmga* transgenic mice also revealed the overexpression of other genes, such as *Ccnb1*, *Ccnd3*, and *Cdc2*, coding for proteins involved in cell cycle regulation (De Martino *et al.* 2007). It is likely that their altered expression may have a role in experimental and human pituitary neoplasias. However, further studies are required to define the role of these proteins in pituitary tumorigenesis.

Concluding remarks

In conclusion, these recent findings provide new insight with respect to the mechanisms that dysregulate the cell cycle in pituitary tumorigenesis. By acting as regulators of different steps of cell cycle progression, the HMGA proteins play a crucial role in the maintenance of the pituitary cell homeostasis. In fact, overexpression of HMGA proteins in mice is sufficient to get a pituitary phenotype only comparable to that observed by the cooperation of two genetic alterations affecting different cell cycle regulators. Also, it is possible that other mechanisms may contribute to the role of HMGA2 in pituitary tumorigenesis, consistent with the ability of HMGA proteins to either activate or repress many different genes.

Since the role of the *HMGA2* overexpression represents a critical event in the development of a significant number of prolactinomas, a therapy based on the suppression of the *HMGA2* function may be

envisaged for the treatment of recurrent prolactinomas. However, as cell cycle alterations can arise independently from the event that has caused them, and they are crucial in pituitary tumorigenesis, therapies aimed to target cell cycle actors may be also conceived. Even not taking into account of the role of HMGA proteins in pituitary cell cycle, transgenic mice overexpressing either *Hmga1* or *Hmga2* may be considered excellent animal models to explore new therapies for pituitary adenomas (Fedele *et al.* 2007).

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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