Int J Clin Exp Pathol 2016;9(3):3245-3255
www.ijcep.com /ISSN:1936-2625/IJCEP0021009

Original Article

Expression of cell-cycle regulators is associated with invasive behavior and poor prognosis in prolactinomas

Chunhui Liu[1,2*], Weiyan Xie[1,2*], Dan Wu[3], Zhenye Li[2], Chuzhong Li[1,2], Yazhuo Zhang[1,2]

[1]Beijing Neurosurgical Institute, Capital Medical University, Beijing, China; [2]Beijing Tiantan Hospital, Capital Medical University, Beijing, China; [3]Department of Neurology, Beijing Renhe Hospital, Beijing, China. [*]Equal contributors.

Received December 2, 2015; Accepted February 13, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Prolactinomas are the most common pituitary tumors. The mechanisms of cell-cycle regulators underlying their invasive biological behavior and poor prognosis have not yet been fully clarified. We classified 48 human prolactinomas as invasive or non-invasive and determined cyclin D1, cyclin E1, p16, p27, Cdk2 and Cdk4 expression by immunohistochemistry analysis of tissue microarray constructs. Then we determined the diagnostic and prognostic value of the cell-cycle regulators expression in human prolactinomas. In this proof of principle study we found that nuclear p16 and p27 expression levels were much lower in invasive prolactinomas compared with non-invasive prolactinomas. Meanwhile, significantly higher cyclin D1 and cyclin E1 expression in invasive prolactinomas compared with normal pituitary or non-invasive prolactinomas. No difference was found in Cdk2 or Cdk4 protein levels in invasive or non-invasive prolactinomas. Regarding clinical outcome, the expression ratios of cyclin D1/p16 and cyclin E1/p27 were significantly positively correlated with clinically inferior outcome ($P<0.001$), while Cdk2 or Cdk4 expression showed no relationship with clinical outcome. Our findings indicate that the expression ratios of cyclin D1/p16 and cyclin E1/p27 are associated with invasion and clinic outcome of prolactinomas. We demonstrate the utility of combined histological analyses of prolactinomas for reliable prediction of tumor invasiveness and recurrence potential.

Keywords: Cell cycle, prolactinomas, neoplasm invasiveness, tissue array analysis

Introduction

Pituitary tumors, which arise from adenohypophyseal cells, are one of the most common intracranial tumors with a prevalence of 1/1,500, with prolactinomas being the most common hormone-secreting pituitary adenomas [1-4]. Pituitary tumors are invariably benign, but cause significant morbidity through mass effects and/or inappropriate secretion of pituitary hormones. Further, pituitary tumors often invade the sphenoid, cavernous sinus, or the dura mater, and can be aggressive, with a high proliferation rate and short time to postoperative recurrence [1-4]. Predicting pituitary tumor behavior remains a challenge [6-8]. In pathological studies, increased levels of Ki-67 and proliferating cell nuclear antigen (PCNA), P53 and pituitary transforming tumor gene (PTTG) have been found in invasive prolactinomas. However, these markers have not yet been correlated with clinical outcome [9-15].

In cancer, the most common mutations occur in cell cycle regulatory genes, potentially leading to uncontrolled tumor growth and progression. Elucidation of cell cycle pathways involved in carcinogenesis may aid cancer management by increasing diagnostic and prognostic accuracy. Several studies have reported the prognostic value of cell cycle regulators in patients with urothelial, breast and adrenal cancers [16-20].

Furthermore, recent molecular analyses of human pituitary neoplasias have revealed deregulation of the cell cycle during pituitary tumorigenesis, as indicated by altered cyclin-dependent kinase (CDK) regulation and suppression of Cdk inhibitory mechanisms [21]. Cyclin D1 and cyclin E1 are often overexpressed in pituitary tumors and exhibit allelic imbalance in

some tumor samples [22, 23]. Cyclin-dependent kinase inhibitor 2A (CDKN2A or $p16^{INK4a}$) and cyclin-dependent kinase inhibitor 1B (CDKN1B or p27) are members of a protein family that specifically inhibits cyclin D-dependent kinases. Cyclin D1-induced activation of CDKs (and in particular Cdk4) causes phosphorylation of retinoblastoma protein (pRB) and subsequent release of E2F transcription factors, which induces expression of genes required for G1/S phase transition [24]. During normal pituitary development, progenitor cell cycle exit is controlled by p27Kip1 in differentiated cells [25]. Recent molecular analysis shows that components of the p16/cyclin D1/Cdk4 or p27/cyclin E1/Cdk2 pathway are frequently altered in pituitary adenomas [26-28]. Despite the critical role of cell-cycle deregulation during pituitary tumorigenesis, the prognostic value of cell-cycle regulators and proliferative markers in terms of prolactinomas aggressiveness and recurrence potential remains unclear.

The objective of the present study was to identify potentially useful markers of biological behavior in prolactinomas. To identify markers of invasion and clinical outcome in pituitary tumors, we used 48 human prolactinomas carefully classified into invasive and non-invasive through radiology using magnetic resonance imaging (MRI) and histology alongside the post-surgical outcome of the 48 patients [29-31]. Patient characteristics (age, sex, preoperative plasma prolactin levels), tumor characteristics (size, invasion, pathological classification), and clinical outcome were assessed from retrospective data with postsurgical follow-up. We selected cell cycle regulators including cyclin D1, cyclin E1, p21, p27, Cdk2 and Cdk4, in an attempt to establish correlations and/or associations with clinical post-surgical outcome.

Patients and methods

Patients

We selected 48 patients who underwent pituitary surgery at Beijing Tiantan Hospital from 2008 to 2012 with plasma PRL levels >200 ng/ml and only PRL immunostaining (plurihormonal prolactin tumors being excluded). Patients include that: 1) Resistant to dopamine; 2) who can't tolerate dopamine therapy. The dopamine resistance was defined as previously published [32]. The medical therapy was interrupted at least 2 months before surgery. This study were reviewed and approved by ethics committee of Beijing Tiantan Hospital affiliated to Capital Medical University (KY2013-015-02). Tumor size was determined by MRI before surgery. Tumors were classified as microadenomas (diameter, <1 cm), macroadenomas (>1 cm and <4 cm), and giant adenomas (>4 cm). Tumor invasion was evaluated from the preoperative MRI for all patients. Postoperative follow-up time ranged from 2.5 to 7 years (mean: 4.8 years). Patients showing no clinical or hormonal (PRL<30 ng/ml) symptoms and no radiological remnant were considered in remission. Persistent disease was defined as increased plasma levels of prolactin with or without a mass visible by radiology. Tumoral recurrence was defined as radiological evidence of tumor regrowth. Tumor grade was based on the following criteria: Invasion was defined as histological and/or radiological (MRI) signs of cavernous or sphenoid sinus invasion. Proliferation was considered positive based on the presence of at least two of the following three criteria: Ki-67 (≥3%); Mitoses: n>2/10 HPF; P53: positive (>10 strongly positive nuclei/10 HPF). The five tumor grades were: Grade 1a: non-invasive tumor; Grade 1b: non-invasive proliferative tumor; Grade 2a: invasive tumor; Grade 2b: invasive and proliferative tumor.

Tumor samples and tissue microarray construction

Formalin-fixed paraffin-embedded tissue blocks were sliced and haematoxylin and eosin stained (H&E) slides were produced. Three core biopsies with a 2.0-mm diameter were selected from the paraffin-embedded tissue. The cores were transferred to TMAs using the Minicore tissue-arraying instrument (Mitogen, UK). Samples were randomly ordered and anonymized on the TMA slides. Tissue microarrays were cut into 4 μm sections using a serial microtome. To minimize loss of antigenicity, the microarray slide was processed within one week of cutting.

Immunohistochemistry (IHC) techniques and antibodies

All TMA slides were evaluated in advance using an H&E stain to assess tumor content and quality. The TMAs were placed in the Leica BOND-III

Table 1. Patient clinical and pathological characteristics

No of patients	Age at Surgery (years)	Sex	Preoperative plasma prolactin levels (g/liter)	Tumor size	Grades	Follow-up time from surgery (years)/outcome
1	48	M	256	Giant	2b	7/persistence
2	30	M	359	Macro	2a	7/recurrence
3	51	M	187	Giant	2a	7/recurrence
4	31	M	320	Giant	2a	6.5/persistence
5	44	F	200	Giant	2a	6/recurrence
6	23	M	330	Giant	2a	6/persistence
7	41	F	258	Giant	2b	4.5/recurrence
8	45	M	320	Giant	2a	6/persistence
9	45	M	5361	Giant	2a	5.5/persistence
10	46	M	252	Macro	2a	4.5/persistence
11	31	M	975	Giant	2a	5/persistence
12	18	M	3193	Giant	2a	4.5/persistence
13	48	M	928	Giant	2b	4.5/recurrence
14	48	F	261	Giant	2a	4/persistence
15	43	F	160	Giant	2a	4/remission
16	40	M	5128	Giant	2a	4/recurrence
17	43	M	181	Giant	2a	4/recurrence
18	46	F	170	Giant	2a	4/recurrence
19	35	M	3705	Giant	2a	4/persistence
20	43	M	2849	Gacro	2a	4/persistence
21	29	F	233	Gacro	2a	4/remission
22	35	F	187	Giant	2a	5/remission
23	14	F	541	Giant	2a	2.5/recurrence
24	34	M	440	Micro	1a	3.5/persistence
25	40	M	160	Macro	1a	6/remission
26	29	F	216	Giant	1a	4/remission
27	27	M	230	Giant	1a	4/persistence
28	43	F	205	Macro	1b	4/remission
29	45	F	197	Macro	1a	5/remission
30	58	M	1076	Macro	1a	5/recurrence
31	30	F	211	Micro	1a	4/remission
32	59	M	899	Macro	1a	5/persistence
33	61	F	167	Macro	1a	5/remission
34	47	M	1288	Macro	1a	5/recurrence
35	33	F	208	Macro	1a	6/remission
36	25	F	174	Micro	1a	6/remission
37	38	F	230	Macro	1a	3/remission
38	31	F	159	Macro	1a	5.5/remission
39	26	M	535	Macro	1a	5.5/persistence
40	46	F	180	Macro	1a	5.5/recurrence
41	47	M	2543	Macro	1a	5.5/recurrence
42	33	F	216	Macro	1a	5.5/remission
43	49	F	170	Macro	1a	5/remission
44	38	M	176	Macro	1a	6/recurrence
45	26	F	178	Macro	1a	3/remission
46	38	F	189	Macro	1a	7/remission
47	62	M	446	Macro	1b	3/persistence
48	45	M	188	Micro	1a	2.5/remission

F, female; M, male.

arrayer (Leica Biosystems, Germany), which is a fully automated, random and continuous access slide-staining system that processes IHC tests simultaneously. Bond™ Polymer Refine Detection HE (Leica Biosystems, Germany) was used for detection of primary antibodies. Immunostains were standardized using appropriate positive and negative controls for each antibody. All TMAs were stained in the same run for each antibody to avoid inter-assay variability. The slides were digitally scanned and expression was examined using Aperio AT2 (Leica Biosystems, Germany). The following antibodies were used: Ki-67 (ab15580, 1/100; Abcam, Cambridge, UK); p53 (ab179477, 1/100; Abcam); Cyclin D1 (ab21699, 1/100; Abcam); Cyclin E1 (ab9517, 1/30; Abcam); p16 (ab54-210, 1/1500; Abcam); p27 (ab32304, 1/500; Abcam); Cdk2 (ab77671, 1/600; Abcam); Cdk4 (ab108357, 1/400; Abcam). The optimal titer of primary antibodies was determined based on pre-experiment results. The results were calculated using Aperio AT2 with digital slide viewing software. The percentage of immunostaining and the staining intensity (0, negative; 1+, weak; 2+, moderate; and 3+, strong) were recorded. An H-score was calculated using the following formula: H-score = (% cells 1+) + 2*(% cells 2+) + 3*(% cells 3+). The maximum H-score was 300, corresponding to 100% of cells with strong intensity.

Statistical analysis

Results are presented as means ± SD or median (inter-

quartile range), depending on data distribution; proportions and frequencies were used for categorical variables. Differences in categorical variables among groups were analyzed by the Chi-squared test. For the comparison of continuous variables one-way ANOVA followed by Newman-Keuls test was used. A *P*-value of ≤0.05 was considered statistically significant.

Results

Patient and tumor characteristics

Patient clinical features are summarized in **Table 1**. The study population was composed of 48 patients (22 women and 26 men). The mean age at surgery was 39.3 ± 10.7 years (range 14-62 years) and the mean preoperative plasma prolactin level was 775.7 g/liter (range, 159-5187 g/liter). Based on the MRI data, 4 (8.3%) patients had microadenomas, 23 (47.9%) had macroadenomas and the remaining 21 (43.8%) had giant adenomas. As shown in **Table 1**, at the end of the follow-up period, 18 patients (37.5%) were in remission, 16 patients (33.3%) had persistent disease, while 14 patients (29.2%) had recurrence. Postoperative follow-up ranged from 2.5-7 years (mean 4.8 years). Fifteen patients with non-invasive tumors (grades 1a and 1b) had gone into remission (15 of 25), 5 had persistent disease, and 5 had recurrent disease. In the invasive subgroup (grades 2a and 2b), 3/23 patients were in remission, 11 had persistent cancer, and 9 had recurrent disease. Therefore, 14 patients (29.2%) had tumors that recurred or progressed under treatment, and 34 patients (70.8%) were considered either cured or in remission at the end of follow-up.

Expression of cyclin D1, cyclin E1, P16, P27, Cdk2 and Cdk4 in normal pituitary and invasive/non-invasive prolactinomas

The results of the immunohistochemical examination of normal pituitary and prolactin pituitary adenomas are summarized in **Figure 1**. For cyclin D1, cyclin E1, p16, and p27, expression levels either incrementally increased or decreased from normal pi-tuitary to invasive prolactin pituitary adenomas. Strong expression of p16 and p27 were consistently noted in nuclei of normal pituitary tissue (mean H-score: 272 and 244, respectively), and there was a consistent but variable reduction in the number of p16- or p27-stained nuclei in tumor tissue (**Figure 1**). Although normal cases showed homogeneous immunostaining, prolactinomas exhibited a heterogeneous pattern of significantly reduced p16 and p27 expression, which was significantly lower in non-invasive prolactinomas compared with normal pituitary (mean H-score: 198 and 165, respectively), with the lowest expression observed in invasive prolactinomas (mean H-score: 154 and 143, respectively) (**Figure 2B**, **2D**).

Cyclin D1 and cyclin E1 were frequently expressed in the nucleus of prolactinomas, while very few normal pituitary tissues showed cyclin D1 and cyclin E1 expression (**Figure 1**). Both the intensity and frequency of cyclin D1 and cyclin E1 staining was much higher in invasive pituitary adenomas than in non-invasive pituitary adenomas (**Figure 2A**, **2C**). As shown in **Figure 2**, expression progressively and significantly increased from normal pituitary to non-invasive prolactinomas to invasive prolactinomas. An inverse association between p16 (or P27) and cyclin D1 (or cyclin E1) expression was observed in prolactinomas.

Cdk2 and Cdk4 immunostains were localized in the cytoplasm and nucleus in both invasive and non-invasive tumors, with no clear difference in the number of stained cells or in staining intensity between the groups (**Figures 1E**, **1F**, **2E**, **2F**).

The prognostic value of clinical characteristics (age, sex and preoperative plasma prolactin levels) on clinical outcome

Univariate statistical analysis of clinical data was performed on the 48 patients for whom complete data were available. By the end of the follow-up period, 18 patients were in remission, 14 had persistence disease and 16 had recurrent disease. Univariate analysis revealed that negative surgical outcome (i.e. persistence or recurrence) was associated with male sex, high preoperative prolactin levels, large tumor size and invasion (**Table 2**).

Diagnostic and prognostic value of the expression ratios of cyclin D1/p16 and cyclin E1/p27 in human prolactinomas

We observed a strong inverse correlation between cyclin E1 up-regulation and p27 down-

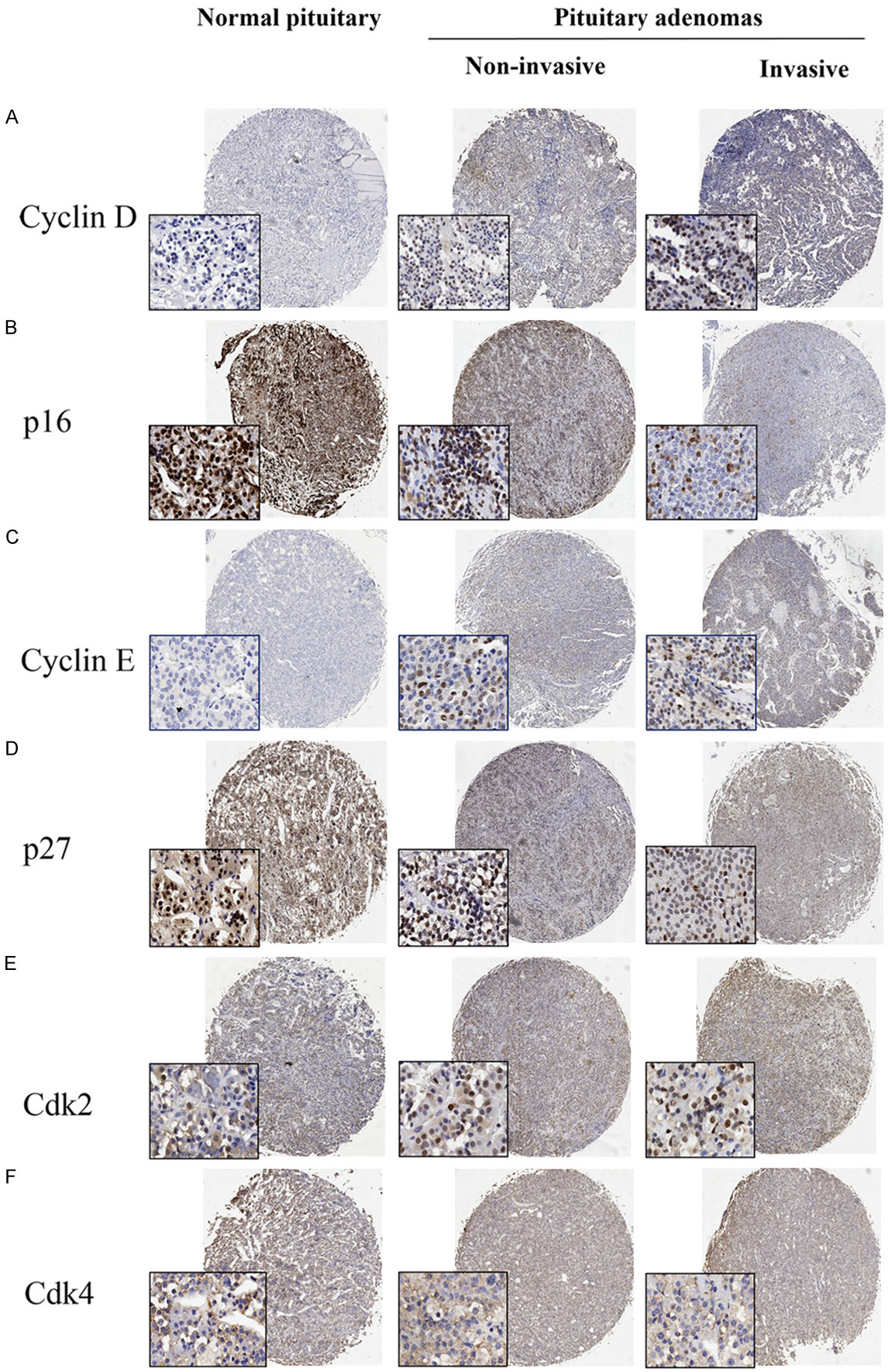
Normal pituitary
Pituitary adenomas
Non-invasive
Invasive
A
Cyclin D
B
p16
C
Cyclin E
D
p27
E
Cdk2
F
Cdk4

Figure 1. Representative images of p16, p27, Cyclin D1, Cyclin E1, Cdk2, and Cdk4 staining on a tissue microarray: Normal pituitary with low Cyclin D1, high p16, low Cyclin E1, high p27 and moderate Cdk2/Cdk4 expression levels (left panel). Non-invasive pituitary adenomas with moderate Cyclin D1, p16, Cyclin E1, p27, Cdk2 and Cdk4 expression levels (middle panel). Invasive pituitary adenomas with high Cyclin D1, low p16, high Cyclin E1, low p27 and moderate Cdk2/Cdk4 expression levels (right panel). The inset shows the 400x magnification of the original images.

regulation, and between cyclin D1 up-regulation and p16 down-regulation in prolactinomas. **Figure 3** shows the strong association between p21, p27, cyclin D1 and cyclin E1 expression and clinicopathological features. The expression ratios of cyclin D1/p16 and cyclin E1/p27 were significantly higher in patients with recurrence than in patients with persistent disease, with the lowest ratio observed in patients in remission (**Figure 3A**, **3B**, $P<0.001$). No differences were observed in the H-score of Cdk2 and Cdk4 expression in patients in remission, patients with persistent or recurrent disease (**Figure 4A**, **4B**).

Discussion

The present study demonstrates that invasive prolactinomas have significantly higher cyclin D1 and cyclin E1, and lower p16 and p27 expression than normal pituitary or non-invasive prolactinomas. We found an inverse association between p16 (or p27) and cyclin D1 (or cyclin E1) expression and these expression ratios were significantly positively correlated with clinically inferior outcomes.

Accurate prognostic factors are necessary for robust clinical decision making, yet current prognostic markers of prolactinomas have limited accuracy. Biomarkers such as cell cycle regulators have the potential to unveil unique biological features thereby identifying patients who are at high risk for adverse outcomes [33].

CDKs and their activating subunits, the cyclins, are essential for proper cell cycle regulation in eukaryotes. Under the regulation of promoters (Cyclin D and E) and CDK inhibitors (p16, p21, p27, p57), CDKs control cell cycle progression through G1/S-phase to DNA synthesis. Shariat et al. have shown the prognostic significance of cell cycle regulators in accurately predicting disease-free survival (DFS) and cancer-specific survival (CSS) in urothelial bladder cancer patients [16]. Youssef et al. demonstrated the utility of a panel of cell cycle regulators in predicting oncologic outcome in patients with squamous cell carcinoma of the bladder [34].

Transgenic overexpression or disruption of cell cycle-associated genes has led to pituitary tumor formation in several animal models [35]. Moreover, aberrant cell proliferation underlies pituitary trophic disorders that lead to pituitary hypoplasia, hyperplasia, or adenoma formation [36, 37]. Pituitary tumors acquire genetic and epigenetic alterations in oncogenes and tumor suppressors that result in unrestrained proliferation, aberrant neuroendocrine regulatory signals and a disrupted humoral milieu, which is mediated directly or indirectly by dysregulated cyclin dependent kinases (CDKs) [38, 39].

In the pituitary, the cell cycle progresses much slower than in skin or digestive tract cells; most adult pro-opiomelanocortin (POMC) cells have exited the cell cycle, do not express detectable cyclin E1, and express p27Kip1 [25]. These findings are consistent with our finding that cyclin D1 and cyclin E1 are almost undetectable in the normal pituitary, while p16 and p27 expression levels are relatively high. However, increased expression of cyclins A, B, D and E has been reported in pituitary adenomas relative to normal pituitary tissue and this change was related to tumor size and re-growth. There is evidence supporting a primary role for cyclin E in cancer, suggesting that deregulation of this protein may play a crucial role in altering G1/S transition, thereby contributing to tumor development. In Cushing's disease cyclin E is preferentially increased in corticotroph adenomas, which may be related to low nuclear p27 levels in these tumors [22]. G1/S transition is the key checkpoint for cell cycle progression and is controlled by cyclin D (in complex with CDK4/6) and cyclin E (in complex with CDK2); cyclin D-CDK4 and cyclin E-CDK2 could be controlled by p16 and p27, respectively [40].

Mice with pituitary-specific cyclin E overexpression (driven by the POMC promoter) develop pituitary hyperplasia and adenomas. When crossed with p27Kip1 knockout mice, these mice have an increased incidence of pituitary tumors, suggesting synergy between cyclin E and p27Kip1 [41]. These results are consistent

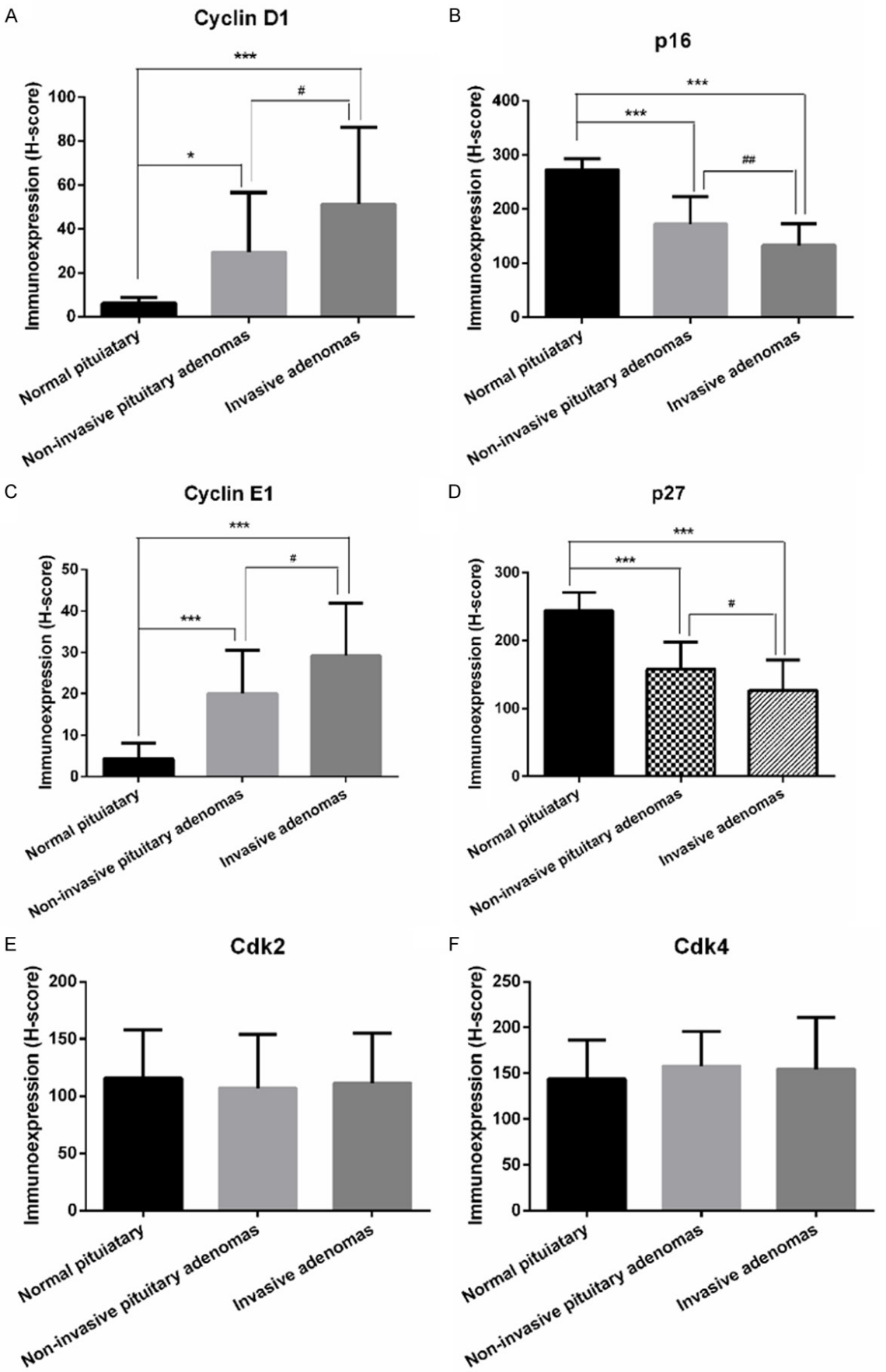
A
Cyclin D1
Immunoexpression (H-score)
0
20
40
60
80
100
***
#
*
Normal pituiatary
Non-invasive pituitary adenomas
Invasive adenomas
B
p16
Immunoexpression (H-score)
0
100
200
300
400
***
***
##
Normal pituiatary
Non-invasive pituitary adenomas
Invasive adenomas
C
Cyclin E1
Immunoexpression (H-score)
0
10
20
30
40
50
***
#
***
Normal pituiatary
Non-invasive pituitary adenomas
Invasive adenomas
D
p27
Immunoexpression (H-score)
0
100
200
300
***
***
#
Normal pituiatary
Non-invasive pituitary adenomas
Invasive adenomas
E
Cdk2
Immunoexpression (H-score)
0
50
100
150
200
Normal pituiatary
Non-invasive pituitary adenomas
Invasive adenomas
F
Cdk4
Immunoexpression (H-score)
0
50
100
150
200
250
Normal pituiatary
Non-invasive pituitary adenomas
Invasive adenomas

Figure 2. Bar graphs represent Cyclin D1 (A), p16 (B), Cyclin E1 (C), p27 (D), Cdk2 (E), and Cdk4 (F) stainings of tissue microarrays. The columns refer to means ± SD. *P<0.05, ***P<0.001, compared with normal pituitary; #P<0.05, ##P<0.01, compared with non-invasive pituitary adenomas. All data were analyzed by ANOVA, followed by the Newman-Keuls multiple comparison test.

Table 2. Clinical and tumor characteristics of patients with Prolactinomas (N=48)

Clinical characteristics	Remission	Persistence	Recurrence	Univariate analysis (*P* value)
No. of patients (%)	18 (37.5%)	16 (33.3%)	16 (39.2%)	
Age (years)	37	39	42	0.4309[a]
Sex				
Females	16	1	5	
Males	2	15	9	5.798e-06[b]
Preoperative plasma prolactin levels (g/liter)	191	660	1658	0.0014[a]
Tumor size				
Micro	3	1	0	
Macro	12	5	6	
Giant	3	10	8	0.0413[b]
Invasive	3	11	9	
Non-invasive	15	5	5	0.0035[b]

a: One-way ANOVA; b: Chi-squared test.

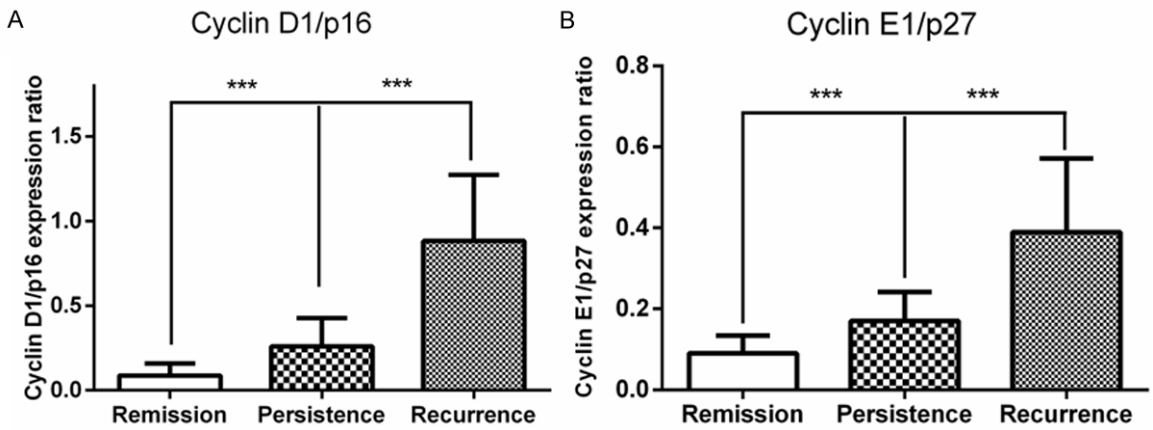

Figure 3. Bar graphs are expressed as the H-score ratio of Cyclin D1/p16 (A), Cyclin E1/p27 (B) expression in patients in remission, patients with persistent or recurrent disease. The columns refer to means ± SD. ***P<0.001, compared with remission group. All data were analyzed by ANOVA, followed by the Newman-Keuls multiple comparison test.

with our finding of a strong inverse correlation between cyclin E1 up-regulation and p27 down-regulation, and between cyclin D1 up-regulation and p16 down-regulation in prolactinomas.

Tumor initiation is a multistep process. Cyclin E expression is essential for cell cycle reentry of quiescent cells-this effect could be suppressed in adult pituitary cells by high levels of p27Kip1, a key regulator of the cyclin E-CDK2 complex. In a multistep model of tumor development, enhanced cyclin E-dependent proliferation may be an early event that makes cells more vulnerable to the effects of a second hit, such as loss of p27Kip1 expression [40]. Once p27 levels decrease, fully active CDK2-cyclin E complexes are available to phosphorylate the Rb protein family, thus allowing cells to progress from the G1 to the S phase of the cell cycle [42]. Individual alterations in cell cycle regulators such as p16, p27, cyclin D1 and cyclin E1 in prolactinomas are unlikely to be sufficient for tumor formation.

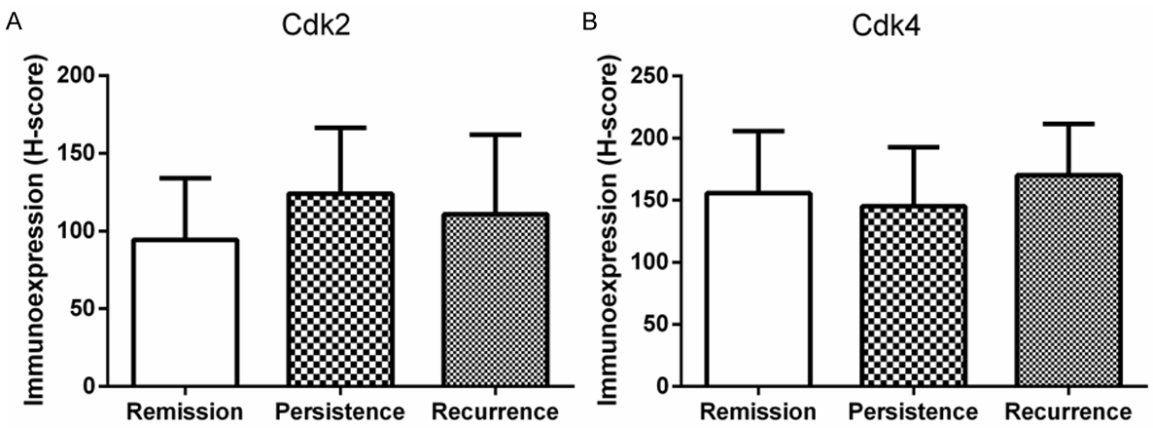

Figure 4. The bar graphs are expressed as the H-score of Cdk2 and Cdk4 expression in patients in remission, patients with persistent or recurrent disease. The columns refer to means ± SD. All data were analyzed by ANOVA, followed by the Newman-Keuls multiple comparison test.

Here, we show that a concomitant increase in cyclin D1/cyclin E1 and decrease in p16/p27 is associated with clinically inferior outcomes. The ratios of cyclin E1 to p27, and cyclin D1 to p27 levels may thus regulate proliferation and act as gatekeeper to protect cells from re-entering the cell cycle. These results highlight the role of cell cycle regulators and the impact of their deregulation in prolactinomas. To our knowledge ours is the first study to evaluate the association of p16 and cyclin D1, and p21 and cyclin E1 expression with oncologic outcomes in patients with prolactinomas.

Acknowledgements

This work were supported by the National High Technology Research and Development Program of China (863 Program) (2014AA020610), the Research Special Fund For Public Welfare Industry of Health (201402008), National Natural Science Foundation of China (31200796), Beijing Nova Program (Z131102000413024).

Disclosure of conflict of interest

None.

Address correspondence to: Yazhuo Zhang, Beijing Neurosurgical Institute, Beijing Tiantan Hospital Affiliated to Capital Medical University, 6 Tiantan Xili, Dongcheng District, Beijing 10050, China. Tel: +86-10-67096765; E-mail: zyz2004520@yeah.net

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: 4769-75.

[2] Fernandez A, Karavitaki N, Wass JA. Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury. Clin Endocrinol (Oxf) 2010; 72: 377-82.

[3] Glezer A, Bronstein MD. Prolactinomas. Endocrinol Metab Clin North Am 2015; 44: 71-8.

[4] Ben-Jonathan N, LaPensee CR, LaPensee E. What can we learn from rodents about prolactin in humans? Endocr Rev 2008; 29: 1-41.

[5] Meij BP, Lopes MB, Ellegala DB, Alden TD, Laws ER Jr. The long-term significance of microscopic dural invasion in 354 patients with pituitary adenomas treated with transsphenoidal surgery. J Neurosurg 2002; 96: 195-208.

[6] Figarella-Branger D, Trouillas J. The new WHO classification of human pituitary tumors: comments. Acta Neuropathol 2006; 111: 71-2.

[7] Grossman AB. The 2004 World Health Organization classification of pituitary tumors: is it clinically helpful? Acta Neuropathol 2006; 111: 76-7.

[8] Melmed S. Pathogenesis of pituitary tumors. Nat Rev Endocrinol 2011; 7: 257-66.

[9] Hsu DW, Hakim F, Biller BM, Monte S, Zervas NT, Klibanski A, Hedley-Whyte ET. Significance of proliferating cell nuclear antigen index in predicting pituitary adenoma recurrence. J Neurosurg 1993; 78: 753-61.

[10] Jaffrain-Rea ML, Stefano D, Minniti G, Esposito V, Bultrini A, Ferretti E, Santoro A, Faticanti Scucchi L, Gulino A, Cantore G. A critical reappraisal of MIB-1 labelling index significance in a large series of pituitary tumours: secreting versus non-secreting adenomas. Endocr Relat Cancer 2002; 9: 103-13.

[11] Knosp E, Kitz K, Perneczky A. Proliferation activity in pituitary adenomas: measurement

by monoclonal antibody Ki-67. Neurosurgery 1989; 25: 927-30.
[12] Levy A, Hall L, Yeudall WA, Lightman SL. p53 gene mutations in pituitary adenomas: rare events. Clin Endocrinol (Oxf) 1994; 41: 809-14.
[13] Daniel L, Trouillas J, Renaud W, Chevallier P, Gouvernet J, Rougon G, Figarella-Branger D. Polysialylated-neural cell adhesion molecule expression in rat pituitary transplantable tumors (spontaneous mammotropic transplantable tumor in Wistar-Furth rats) is related to growth rate and malignancy. Cancer Res 2000; 60: 80-5.
[14] Trouillas J, Daniel L, Guigard MP, Tong S, Gouvernet J, Jouanneau E, Jan M, Perrin G, Fischer G, Tabarin A, Rougon G, Figarella-Branger D. Polysialylated neural cell adhesion molecules expressed in human pituitary tumors and related to extrasellar invasion. J Neurosurg 2003; 98: 1084-93.
[15] Zhang X, Horwitz GA, Heaney AP, Nakashima M, Prezant TR, Bronstein MD, Melmed S. Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. J Clin Endocrinol Metab 1999; 84: 761-7.
[16] Shariat SF, Chade DC, Karakiewicz PI, Ashfaq R, Isbarn H, Fradet Y, Bastian PJ, Nielsen ME, Capitanio U, Jeldres C, Montorsi F, Lerner SP, Sagalowsky AI, Cote RJ, Lotan Y. Combination of multiple molecular markers can improve prognostication in patients with locally advanced and lymph node positive bladder cancer. J Urol 2010; 183: 68-75.
[17] Chatterjee SJ, Datar R, Youssefzadeh D, George B, Goebell PJ, Stein JP, Young L, Shi SR, Gee C, Groshen S, Skinner DG, Cote RJ. Combined effects of p53, p21, and pRb expression in the progression of bladder transitional cell carcinoma. J Clin Oncol 2004; 22: 1007-13.
[18] Zini L, Porpiglia F, Fassnacht M. Contemporary management of adrenocortical carcinoma. Eur Urol 2011; 60: 1055-65.
[19] Duregon E, Volante M, Giorcelli J, Terzolo M, Lalli E, Papotti M. Diagnostic and prognostic role of steroidogenic factor 1 in adrenocortical carcinoma: a validation study focusing on clinical and pathologic correlates. Hum Pathol 2013; 44: 822-8.
[20] Jonat W, Arnold N. Is the Ki-67 labelling index ready for clinical use? Ann Oncol 2011; 22: 500-2.
[21] Malumbres M, Barbacid M. To cycle or not to cycle: a critical decision in cancer. Nature Rev Cancer 2001; 1: 222-31.
[22] Jordan S, Lidhar K, Korbonits M, Lowe DG, Grossman AB. Cyclin D and cyclin E expression in normal and adenomatous pituitary. Eur J Endocrinol 2000; 143: R1-R6.
[23] Hibberts NA, Simpson DJ, Bicknell JE, Broome JC, Hoban PR, Clayton RN, Farrell WE. Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 1999; 5: 2133-9.
[24] Harbour JW, Dean DC. The Rb/E2F pathway: expanding roles and emerging paradigms. Genes Devel 2000; 14: 2393-409.
[25] Bilodeau S, Roussel-Gervais A, Drouin J. Distinct developmental roles of cell cycle inhibitors p57Kip2 and p27Kip1 distinguish pituitary progenitor cell cycle exit from cell cycle reentry of differentiated cells. Mol Cell Biol 2009; 29: 1895-908.
[26] Yoshino A, Katayama Y, Ogino A, Watanabe T, Yachi K, Ohta T, Komine C, Yokoyama T, Fukushima T. Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas. J Neurooncol 2007; 83: 153-62.
[27] Tani Y, Inoshita N, Sugiyama T, Kato M, Yamada S, Shichiri M, Hirata Y. Upregulation of CDKN2A and suppression of cyclin D1 gene expressions in ACTH-secreting pituitary adenomas. Eur J Endocrinol 2010; 163: 523-9.
[28] Muşat M, Morris DG, Korbonits M, Grossman AB. Cyclins and their related proteins in pituitary tumourigenesis. Mol Cell Endocrinol 2010; 326: 25-9.
[29] Gürlek A, Karavitaki N, Ansorge O, Wass JA. What are the markers of aggressiveness in prolactinomas? Changes in cell biology, extracellular matrix components, angiogenesis and genetics. Eur J Endocrinol 2007; 156: 143-53.
[30] Knosp E, Steiner E, Kitz K, Matula C. Pituitary adenomas with invasion of the cavernous sinus space: a magnetic resonance imaging classification compared with surgical findings. Neurosurgery 1993; 33: 610-7.
[31] Raverot G, Vasiljevic A, Jouanneau E, Trouillas J. A prognostic clinicopathologic classification of pituitary endocrine tumors. Endocrinol Metab Clin North Am 2015; 44: 11-8.
[32] Delgrange E, Sassolas G, Perrin G, Jan M, Trouillas J. Clinical and histological correlations in prolactinomas, with special reference to bromocriptine resistance. Acta Neurochirurgica 2005; 147: 751-8.
[33] Karam JA, Lotan Y, Karakiewicz PI, Ashfaq R, Sagalowsky AI, Roehrborn CG, Shariat SF. Use of combined apoptosis biomarkers for prediction of bladder cancer recurrence and mortality after radical cystectomy. Lancet Oncol 2007; 8: 128-36.
[34] Youssef R, Kapur P, Shariat SF, Arendt T, Kabbani W, Mosbah A, Abol-Enein H, Ghoneim M, Lotan Y. Prognostic value of apoptotic mark-

ers in squamous cell carcinoma of the urinary bladder. BJU Int 2012; 110: 961-6.
[35] Melmed S. Pituitary tumors. Endocrinol Metab Clin North Am 2015; 44: 1-9.
[36] Farrell WE. Pituitary tumours: Findings from whole genome analyses. Endocr Relat Cancer 2006; 13: 707-16.
[37] Scheithauer BW, Gaffey TA, Lloyd RV, Sebo TJ, Kovacs KT, Horvath E, Yapicier O, Young WF Jr, Meyer FB, Kuroki T, Riehle DL, Laws ER Jr. Pathobiology of pituitary adenomas and carcinomas. Neurosurgery 2006; 59: 341-53.
[38] Moons DS, Jirawatnotai S, Parlow AF, Gibori G, Kineman RD, Kiyokawa H. Pituitary hypoplasia and lactotroph dysfunction in mice deficient for cyclin-dependent kinase-4. Endocrinology 2002; 143: 3001-8.
[39] Melmed S. Acromegaly pathogenesis and treatment. J Clin Invest 2009; 119: 3189-202.
[40] Galderisi U, Jori FP, Giordano A. Cell cycle regulation and neural differentiation. Oncogene 2003; 22: 5208-19
[41] Roussel-Gervais A, Bilodeau S, Vallette S, Berthelet F, Lacroix A, Figarella-Branger D, Brue T, Drouin J. Cooperation between cyclin E and p27 (Kip1) in pituitary tumorigenesis. Mol Endocrinol 2010; 24: 1835-45.
[42] Reed SI. Ratchets and clocks: The cell cycle, ubiquitylation and protein turnover. Nat Rev Mol Cell Biol 2003; 4: 855-864.