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Functional Inactivation of pRb Associated with Cyclin D1- and Cyclin-dependent Kinase 4 Overexpression Plays A Key Role in Human Pituitary Tumorigenesis

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Background : Human pituitary adenoma (PA) is a common intracranial tumor, but the mechanism underlying tumorigenesis has not been established. Functional inactivation of retinoblastoma protein (pRb) following cyclin D1- and cyclin-dependent kinase (CDK) 4-dependent hyperphosphorylation is one of the most important mechanisms in tumor cell proliferation. We evaluated immunohistochemical expressions of cyclin D1, CDK4 and phosphorylated pRb (p-pRb) in 50 PAs to investigate a role for functional inactivation of pRb associated with cyclin D1/CDK4 overexpression in pituitary tumorigenesis and to correlate it with clinicopathologic variables. **Methods :** Fifty human PAs were immunohistochemically stained for cyclin D1, CDK4 and p-pRb (Thr 356). Correlations between their expression and the clinicopathologic characteristics were statistically analyzed. **Results :** Cyclin D1 and CDK4 were overexpressed in 56% and 64%, respectively; pRb was hyperphosphorylated in 64%. Forty one cases (82%) showed one or more of these altered expressions. Overexpressions of cyclin D1 and CDK4 were correlated with functional pRb inactivation. Cyclin D1 overexpression was associated with apoplexy and growth hormone production. **Conclusions :** Functional inactivation of pRb associated with the cyclin D1/CDK4 overexpression might play a key role in human pituitary tumorigenesis. CDK4 worked in concert with cyclin D1 to hyperphosphorylate pRb. Pituitary apoplexy appeared to be associated with cyclin D1 overexpression.

Key Words : Pituitary adenoma; Retinoblastoma protein; Cyclin D1; Cyclin-dependent kinase 4; Immunohistochemistry

Human pituitary adenoma (PA) is a common benign neoplasm of the central nervous system, which has been reported to encounter in about 10-15% of all intracranial tumors.[1] However, the molecular mechanism of pituitary tumorigenesis has not yet been fully elucidated. Previous studies, especially on the cell cycle regulation, have shown that deregulation of the cyclin D/cyclin-dependent kinases (CDK)/CDK inhibitor /pRb pathway in the G_1/S phase may represent an obligatory step in pituitary tumorigenesis.[2,3] Progression through G_1 to the S phase of the cycle is mediated by the interplay between the proteins that control pRb phosphorylation. Under mitogenic stimulation, the cyclin D1 binds to CDK4, which is subsequently activated and phosphorylates pRb. In a hyperphosphorylated form, pRb is inactivated and the cells are released from G_1 arrest.[4] A few reports on the cyclin D1 overexpression and its genetic alteration in PA have suggested that the overexpression of cyclin D1 occurs early and late in pituitary tumorigenesis and this is not necessarily associated with cyclin D1 gene (CCND1) allelic imbalance.[4-6] There have only been rare studies concerned with CDK4, because only a few studies have reported that amplification of the CDK4 gene was not detected in PAs.[2,3] No data on CDK4 protein in human PAs has been accumulated to date. The previous studies on the genetic alterations of RB have been controversial, with the findings ranging from no or rare allelic loss to promoter methylation or allelic deletion in PAs.[3,7-9] Therefore, primary RB inactivation seems to be uncommon in PAs, and the mechanisms responsible for pRb loss, which occurs infrequently but tends to be associated with the tumor subtype (i.e. somatotropinoma),[5,10] have not yet been clarified.

In addition to the loss of pRb itself, hyperphosphorylation of pRb due to cyclin D1 or CDK4 may lead to uncontrolled cellular proliferation, a hallmark of tumorigenesis.[11-13] The correlation between cell proliferation and pRb phosphorylation suggests that the ability of pRb to constrain cell cycle progression

is inhibited by phosphorylation.[14] The majority of the human CDK phosphorylation sites in pRb have been reported to be involved in the regulation of RB-E2F interaction, including the sites Thr252, Thr356, Thr821, Thr826, Ser780, Ser788, Ser795, Ser807, Ser811 etc.[15] However, there has been no report about the role of functional inactivation of pRb by its hyperphosphorylation in pituitary tumorigenesis. The overexpressions of cyclin D1 and CDK4 have also never been studied in combination with pRb hyperphosphorylation in human PAs. The clinicopathologic correlation between these cell cycle regulators and the biologic behavior of PAs has not been examined much, although there is some data for their association with the hormonal functions of PA. Therefore, we investigated the immunohistochemical expressions of cyclin D1, CDK4 and phosphorylated pRb (p-pRb) in 50 PAs to establish the role of the functional inactivation of pRb associated with the cyclin D1/CDK4 overexpression in pituitary tumorigenesis, and we correlated this with the clinicopathologic variables.

MATERIALS AND METHODS

Patients and tissue samples

Fifty cases of pituitary adenoma were obtained from 47 patients who underwent tumor excision by a transsphenoidal approach (43 patients) or by craniotomy (4 patients) at Dankook University Hospital between 1996 and 2007. Forty-five primary and 5 recurrent tumors were included in this study, and two of the recurrent cases were operated on at this institute initially as recurrent PAs. Three recurrent PA samples were examined along with each corresponding primary tumor, and the intervals between the first and the second operation ranged from 2 to 5 years. Tumors were graded according to a modified Hardy classification.[16] Grade 1 tumors were microadenomas (<1 cm in diameter), and grade 2 tumors were enclosed macroadenomas (>1 cm in diameter) with or without suprasellar extension. Both the grade 1 and grade 2 tumors were considered to be noninvasive. Grade 3 tumors were locally invasive with evidence of bony destruction and tumor within the sphenoid and/or cavernous sinus. Grade 4 tumors demonstrated central nervous system/extracranial spreading with or without metastases. Both the grade 3 and 4 tumors were considered to be invasive. Clinical data such as age, gender, pituitary apoplexy, recurrence, and radiation therapy were also obtained from the medical records of the 47 patients.

Immuohistochemical analysis

The standard avidin-biotin-peroxidase complex method using the LSAB kit (DAKO, Glostrup, Denmark) was used for immunohistochemical staining with monoclonal antibodies against human cyclin D1 (SP4, NeoMarkers, Fremont, CA, USA), human CDK4 (C-22:sc-260, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and human p-pRb (pT- 356:EPR2153AY, Epitomics, Burlingame, CA, USA). The 4 μm-thick tissue sections were deparaffinized with standard xylene and they were hydrated through a series of graded alcohol solutions. The sections were microwaved in 10 mM citrate buffer at 90°C for 10 min and then treated with 3% H_2O_2-PBS solution to reduce the endogenous peroxidase activity. Then they were incubated with normal bovine serum to reduce the nonspecific antibody binding and they were subsequently subjected to the primary antibody reactions. The primary antibodies for the cyclin D1, CDK4 and p-pRb were reacted with the sections at room temperature for one hour at the dilution of 1:100, 1:400, and 1:100, respectively. Negative controls (pituitary tumors with the primary antibody omitted) were used for each series of staining. Human palatine tonsils for cyclin D1, normal human skin for CDK4 and human lung carcinoma for p-pRb were used as each positive control tissue, respectively. The sections were subjected to a color reaction with 3,3-diaminobenzidine tetrahydrochloride that contained 3% H_2O_2 in Tris buffer. They were lightly counterstained with Mayer's hematoxylin.

For evaluating the expressions of cyclin D1, CDK4 and p-pRb, the tumor cells were considered positive only when distinct nuclear staining was seen, regardless of the associated cytoplasmic staining, which was faint and considered non-specific. The cases were considered to be positive for cyclin D1 and CDK4 proteins, respectively, when more than 10% of tumor cells showed distinct nuclear positivity.[2] The positive cases for cyclin D1 and CDK4 were interpreted as cyclin D1 and CDK4 overexpression, respectively. Cases were regarded as being hyperphosphorylated (or positive) for p-pRb if either more than 5% of the tumor cell nuclei showed a distinct signal with moderate to strong intensity or more than 10% of the nuclei were stained recognizable but more obvious as compared to the internal normal controls.[16] Those cases with the positive immunostaining for p-pRb were considered to have functional pRb inactivation due to hyperphosphorylation at threonine 356.[15]

Statistical analysis

Comparison of the immunoreactivity between two-categori-

cal clinicopathologic variables and the relationships between the altered expressions of cyclin D1, CDK4 and p-pRb were analyzed by chi-square tests (SPSS 15.0, Chicago, IL, USA). A p-value less than 0.05 was defined as statistically significant.

RESULTS

Immunoexpression patterns of cyclin D1, CDK4 and p-pRb in 50 human pituitary adenomas

These G_1/S cell cycle regulator proteins showed distinct immunostaining specifically in the nuclei and not in the cytoplasm of the adenoma cells (Fig. 1-3). Normal entrapped pituitary glands or stromal tissues showed neither cyclin D1 nor CDK4 immunopositivity. A p-pRb immunoexpression was never or only rarely found in the internal control tissues. The overexpression of cyclin D1 and CDK4 was demonstrated in 28 (56%) and 32 (64%) of the 50 PAs, respectively. Positive p-pRb staining was found in 32 (64%) of the cases, when the positive cases were interpreted to have the functionally inactivated pRb by its hyperphosphorylation. One or more of these instances of altered expression was observed in 41 cases (82%).

Inter-relationships between the altered expressions for cyclin D1, CDK4 and pRb (Table 1)

Cyclin D1 overexpression was significantly correlated with p-pRb expression (p=0.038) (Table 1-A). p-pRb expression was tightly correlated with CDK4 expression (p=0.020) (Table 1-B).

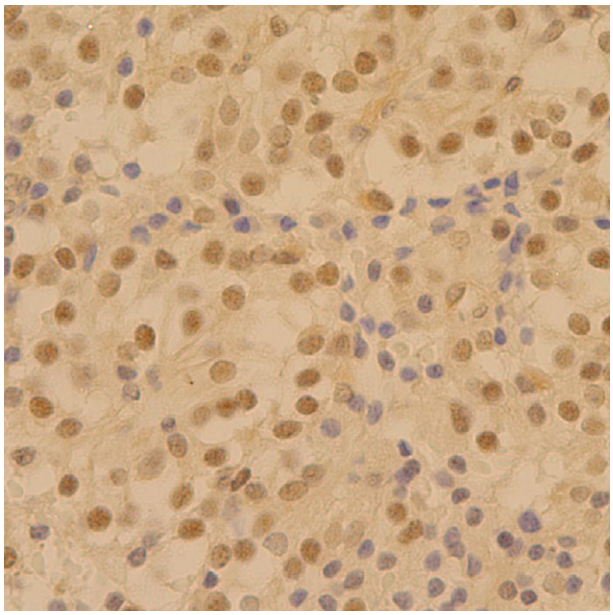

Fig. 2. Cyclin-dependent kinase 4 protein is immunohistochemically overexpressed in most of the tumor cells compared to negative stromal cells.

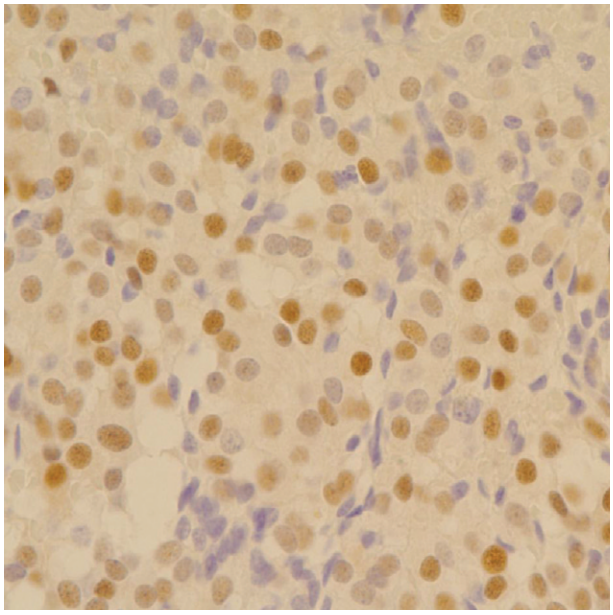

Fig. 1. Immunohistochemical staining for cyclin D1 reveals diffuse nuclear reactivity in pituitary adenoma cells compared to negative internal controls such as the intervening stroma.

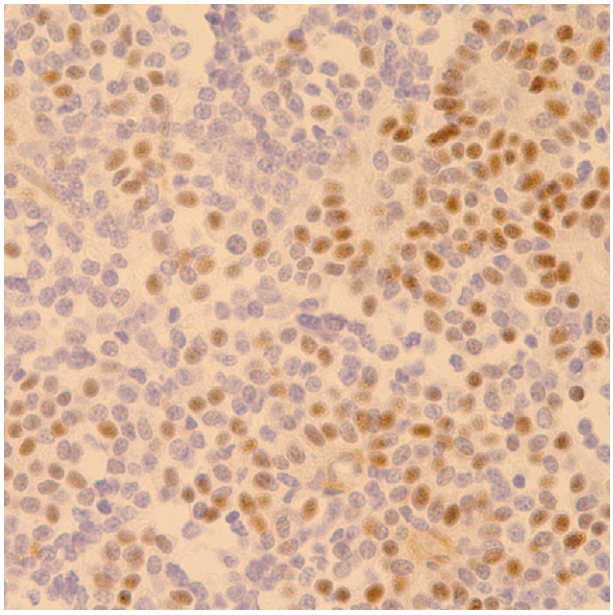

Fig. 3. Immunostaining for phosphorylated pRb discloses hyperphosphorylation of pRb, with its distinct nuclear reactivity in many pituitary adenoma cells.

Table 1. Inter-relationships between altered expression for cyclin D1, Cdk4, and p-pRb proteins in 50 human pituitary adenomas

1-A. Correlation between functional inactivation of pRb and cyclin D1 overexpression

Functional inactivation of pRb	No. of cases	Cyclin D1 overexpression		p-value
		Positive	Negative	
Positive	32	24 (75)	8 (25)	0.038
Negative	18	8 (44)	10 (56)	

1-B. Correlation between functional inactivation of pRb and Cdk4 overexpression

Functional inactivation of pRb	No. of cases	Cdk4 overexpression		p-value
		Positive	Negative	
Positive	32	22 (69)	10 (31)	0.020
Negative	18	6 (33)	12 (67)	

1-C. Correlation between cyclin D1 overexpression and Cdk4 overexpression

Cyclin D1 overexpression	No. of cases	Cdk4 overexpression		p-value
		Positive	Negative	
Positive	32	21 (66)	11 (34)	0.083
Negative	18	7 (39)	11 (61)	

However, correlation between the cyclin D1 and CDK4 overexpressions was not evident, but this seemed to be marginally significant (p=0.083) (Table 1-C). These results showed the functional inactivation of pRb by its hyperphosphorylation was statistically clearly associated with the cyclin D1 and CDK4 overexpressions in human PAs.

Clinicopathologic data and its relationship with a solitary or combined overexpression of cyclin D1, CDK4 and p-pRb

The data is summarized in Table 2. This study cohort consisted of 22 males and 25 females. The average age of the males was 47.6 years and that for the females was 43.8 years. The combined average age was 45.6 years. Of the 50 PAs, 26 were classified as clinically non-functioning based on the medical records and 24 as functioning adenomas (PRL, GH, ACTH, TSH and ADH). The functioning adenomas secreted the hormones of prolactin (PRL, 14), growth hormone (GH, 7), adrenocorticotropic hormone (ACTH, 5), thyroid stimulating hormone (TSH, 2)

Table 2. Correlations between clinicopathologic findings and altered expression for cyclin D1, Cdk4, and p-pRb proteins in 50 human pituitary adenomas

Clinicopathologic characteristics	Cyclin D1 overexp.		Cdk4 overexp.		pRb hyperphosph.	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Age						
≤45 years (n=30)	18 (60)	12 (40)	18 (60)	12 (40)	17 (57)	13 (43)
>45 years (n=20)	14 (70)	6 (30)	10 (50)	10 (50)	15 (75)	5 (25)
Sex						
Male (n=23)	15 (65)	8 (35)	12 (52)	11 (48)	13 (57)	10 (43)
Female (n=27)	17 (63)	10 (37)	16 (59)	11 (41)	19 (70)	8 (30)
Recurrence						
Primary (n=45)	28 (62)	17 (38)	25 (56)	20 (44)	28 (62)	17 (38)
Recurrent (n=5)	4 (80)	1 (20)	3 (60)	2 (40)	4 (80)	1 (20)
Hormonal function						
N-FNL (n=26)	18 (69)	8 (31)	14 (54)	12 (46)	19 (73)	7 (17)
FNL (n=24)	14 (58)	10 (42)	14 (58)	10 (42)	13 (54)	11 (46)
PRL production						
Yes (n=14)	10 (71)	4 (29)	9 (64)	5 (46)	7 (50)	7 (50)
No (n=36)	22 (61)	14 (39)	19 (53)	17 (47)	25 (69)	11 (31)
GH production						
Yes (n=7)	1 (14)	6 (86)*	3 (43)	4 (57)	3 (43)	4 (57)
No (n=43)	31 (72)	12 (28)	25 (58)	18 (42)	29 (67)	14 (33)
Grade (invasion)						
1-2 (N-inv) (n=42)	27 (64)	15 (36)	23 (55)	19 (45)	17 (40)	25 (60)
3-4 (Inv) (n=8)	5 (63)	3 (37)	5 (63)	3 (37)	4 (50)	4 (50)
P. apoplexy						
Present (n=7)	7 (100)†	0 (0)	5 (71)	2 (29)	1 (14)	6 (86)
Absent (n=43)	25 (58)	18 (42)	23 (53)	20 (47)	20 (47)	23 (53)

*,†, p-value<0.05.

overexp., overexpression; hyperphosp., hyperphosphorylation; N-FNL, nonfunctional; FNL, functional; PRL, prolactin; GH, growth hormone; N-inv, noninvasive; Inv, invasive; P., pituitary.

and antidiuretic hormone (ADH, 1). Among these, two cases showed mixed PRL and GH production, two mixed ACTH and TSH, and one mixed ACTH and ADH. According to a modified Hardy classification, 42 PAs (84%) were non-invasive with grade 1 or 2, and they usually showed micro- or macroadenomas (>1 cm) located within the sella turcica. Eight tumors (16%) were invasive with grade 3, and these tumors showed invasion to the cavernous or sphenoid sinus. There were no grade 4 tumors in this tumor cohort. Pituitary apoplexy, which is characterized by massive hemorrhagic infarct within the tumor, occurred in 7 cases (14%).

All 7 tumors showing pituitary apoplexy displayed a cyclin D1 overexpression (100%), whereas 43 tumors with no pituitary apoplexy had 58% of cyclin D1 immunoreactivity (p=0.040). Most tumors with GH production (6/7, 86%) showed no or very low cyclin D1 immunoreactivity, and this was found to be inversely correlated with cyclin D1 overexpression (p=0.003). Each of the CDK4 and p-pRb expressions, as well as any combined expressions, never exhibited a significant association with the clinicopathologic variables.

DISCUSSION

The transition from G_1 to S phase of the cell cycle is known to be regulated by mediators that result in pRb phosphorylation. Deregulation of this pathway has been extensively studied in numerous tumor types, as it might play a key role in tumorigenesis.[18-20] In human pituitary adenomas, the alterations in G_1/S cell cycle regulation have not been extensively studied, so there are no data on CDK4 protein and phosphorylated pRb (p-pRb), although some data for cyclin D1 or E genes/protein and the genes for CDK4 and pRb has been accumulated to a degree.[2-9]

According to the literature,[2-6] it has generally been reported that a cyclin D1 overexpression occurs at a relatively high frequency (49-54%) when detected by an immunohistochemical method and it is not correlated with the tumor grade or an allelic imbalance of the CCND1 gene.[2,4] Therefore, recent studies have described a possible role for cyclin D1 overexpression, which may be an early event in pituitary tumorigenesis.[4,5] The data on CDK4 gene alterations has failed to reveal any consistent amplification or point mutations, and there are no reports on the CDK4 expression in human PAs.[2,3] Our findings such as the relatively high frequency of cyclin D1 and CDK4 overexpressions and the lack of a statistically significant difference in the frequency of these overexpressions between primary and recurrent adenomas suggest that these alterations occur early and they might be required events in human pituitary tumorigenesis. To best of our knowledge, this is the first report that a CDK4 overexpression might be a key component in pituitary tumorigenesis via the deregulation of G_1/S cell cycle control. Deregulation of cyclin D1 synthesis has been reported to allow cell cycle progression in the absence of growth factors and may contribute to the initiation of oncogenesis.[21]

Since RB gene inactivation is unlikely to play a role in pituitary tumorigenesis[8,9] and shows no association with the loss of pRb, functional inactivation of pRb by its hyperphosphorylation could be the next topic of the study for pituitary tumorigenesis via the pRb pathway. In fact, it has been reported that hyperphosphorylation of pRb due to cyclin D1 or CDK4 may lead to uncontrolled cellular proliferation.[11-13] The biological significance of pRb hyperphosphorylation and its impact on the ordered progression through G_1/S are exemplified by the amplification of the cyclin D1 gene in breast and esophageal cancer cells, which is believed to promote pRb phosphorylation.[22] The majority of the CDK phosphorylation sites in pRb are known to be involved in the regulation of RB-E2F interaction, including the sites Ser795, Ser811, Ser807, Thr821, Thr356 etc.[15] The primary anti-Rb phospho (pT356) antibody used in this study detected the increase in phosphorylated pRb (p-pRb) in the tumor cell nuclei in 32 (64%) of the 50 PAs. This immunostaining result suggests that hyperphosphorylated pRb plays an important role in human PAs via inactivating the growth suppression function of pRb.

Our data show that 75% and 79% of the tumors that overexpressed cyclin D1 and CDK4, respectively, also overexpressed p-pRb, with each correlation between the cyclin D1/CDK4 overexpression and the p-pRb expression (p=0.038 and 0.020) being statistically significant. These findings are consistent with the concensus that the overexpression of cyclin D1 leads to the increased phosphorylation of pRb,[18,23] although no data on CDK4 overexpression has yet been accumulated. Thus, the functional inactivation of pRb following cell cycle-dependent hyperphosphorylation by the cyclin D1/CDK4 complex might play a key role in human pituitary tumorigenesis.

Aberrations of one or more components of the pRb/cyclin D1/CDK4 pathway have been reported to occur as frequent events, with a reported frequency of 80-82% in sporadic human PAs.[2,3] In this study, 41 (82%) of the 50 PAs showed altered expressions of one or more proteins (cyclin D1, CDK4 and pRb), demonstrating a marked alteration in the RB1 pathway in human PAs.

The consistent results for the clinicopathologic correlation that have been hitherto reported have shown that a cyclin D1 overexpression or deregulation of either component (pRb, cyclin D1 or p16) of the G_1/S pathway is statistically more frequent in non-functioning and aggressive (or invasive) PAs than in somatrophinomas or other functioning tumors. The loss of RB1 or pRb has been reported to be found at a higher frequency in invasive tumors or somatrophinomas.[10,24] However, our results showed no correlation between most of the clinicopathologic variables and any component of the cyclin D1/CDK4/pRb pathway, either alone or in combination, except for pituitary apoplexy and growth hormone (GH) secretion (Table 1). Pituitary apoplexy was found to be more frequent in the cyclin D1-positive cases, whereas GH production was observed in more of the cyclin D1-negative cases. These results appear to be contradictory, because pituitary apoplexy is most often seen in GH- and ACTH- producing adenomas. Because only 7 PAs each showing pituitary apoplexy and GH production were analyzed in this study, it will be necessary to collect the data from more cases to clear up this discrepancy.

In conclusion, the increased p-pRb expression in human PAs was tightly correlated with the cyclin D1/CDK4 overexpressions, suggesting that functional inactivation of pRb associated with cyclin D1- and CDK4 overexpressions might play a key role in human pituitary tumorigenesis. Moreover, as the vast majority of the pituitary adenomas (82%) displayed one or more alterations in the pRb/cyclin D1/CDK4 pathway, this regulatory pathway may be useful, intriguing target for some diagnostic or therapeutic approaches. Pituitary apoplexy tended to occur in the tumors with cyclin D1 overexpression, although the mechanism underlying this correlation must be further examined.

REFERENCES

1. Asa SL, Ezzat S. The cytogenesis and pathogenesis of pituitary adenomas. Endocr Rev 1998; 19: 798-827.
2. Simpson DJ, Frost SJ, Bicknell JE, *et al*. Aberrant expression of G_1/S regulators is a frequent event in sporadic pituitary adenomas. Carcinogenesis 2001; 22: 1149-54.
3. Ogino A, Yoshinno A, Katayama Y, *et al*. The $p15^{INK4b}$/$p16^{INK4a}$/RB1 pathway is frequently deregulated in human pituitary adenomas. J Neuropathol Exp Neurol 2005; 64: 398-403.
4. Hibberts NA, Simpson DJ, Bicknell JE, *et al*. Analysis of cyclin D1 (CCND1) allelic imbalance and overexpression in sporadic human pituitary tumors. Clin Cancer Res 1999; 5: 2133-9.
5. Farrell WE, Clayton RN. Molecular genesis of pituitary tumors. Frontiers in Neuropathol 2000; 21: 174-98.
6. 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: R1-6.
7. Cryns V, Alexander JM, Klibanski A, Arnold A. The retinoblastoma gene in human pituitary tumors. J Clin Endocrinol Metab 1993; 77: 644-6.
8. Bates AS, Farrell WE, Bicknell EJ, *et al*. Allelic deletion in pituitary adenomas reflects aggressive biological activity and has potential value as a prognostic marker. J Clin Endocrinol Metab 1997; 82: 818-24.
9. Woloschak M, Yu A, Xiao J, Post KD. Abundance and state of phosphorylation of the retinoblastoma gene product in human pituitary tumors. Int J Cancer 1996; 67: 16-9.
10. Simpson DJ, Magnay J, Bicknell JE, *et al*. Chromosome 13q deletion mapping in pituitary tumors: infrequent loss of the retinoblastoma susceptibility gene (RB1) locus despite loss of RB1 protein product in somatotrophinomas. Cancer Res 1999; 59: 1562-6.
11. Dowdy SF, Hinds PW, Louie K, Reed SL, Arnold A, Weinberg RA. Physical interaction of the retinoblastoma protein with human D cyclins. Cell 1993; 73: 499-511.
12. Serrano M, Lee HW, Chin L, Cordon-Cardo C, Beach D, DePinho RA. Role of the INK4A locus in tumor suppression and cell mortality. Cell 1996; 85: 27-37.
13. Sherr CJ. D type cyclins. Trends Biochem Sci 1995; 20: 187-90.
14. Connell-Crowley L, Harper JW, Goodrich DW. Cyclin D1/Cdk4 regulates retinoblastoma protein-mediated cell cycle arrest by site-specific phosphorylation. Mol Biol Cell 1997; 8: 287-301.
15. Knudsen ES, Wang JY. Dual mechanisms for the inhibition of E2F binding to RB by cyclin-dependent kinase-mediated RB phosphorylation. Mol Cell Biol 1997; 17: 5771-83.
16. Roesch A, Becker B, Meyer S, *et al*. Overexpression and hyperphosphorylation of retinoblastoma protein in the progression of malignant melanoma. Mod Pathol 2005; 18: 565-72.
17. Hardy J. Transsphenoidal microsurgical treatment of pituitary tumours. In: Linfoot J, ed. Recent advances in the diagnosis and treatment of pituitary tumours. New York: Raven Press, 1979; 375-88.
18. Betticher DC, White GR, Vonlanthen S, *et al*. G1 control gene status is frequently altered in resectable non-small cell lung cancer. Int J Cancer 1997; 74: 556-62.
19. Roncalli M, Bosari S, Marchetti A, *et al*. Cell cycle related gene abnormalities and product expression in oesophageal carcinoma. Lab Invest 1998; 78: 1049-57.
20. Palmero I, Peters G. Perturbation of cell cycle regulators in human cancer. Cancer Surv 1996; 27: 351-67.
21. Asa SL, Ezzat S. The cytogenesis and pathogenesis of pituitary ade-

nomas. Endocr Rev 1998; 19: 798-827.
22. Sherr CJ. Cancer cell cycles. Science 1996; 274: 1672-7.
23. Betticher DC, Heighway J, Hasleton PS, *et al*. Prognostic significance of CCND1 (cyclin D1) overexpression in primary non-small cell lung cancer. Br J Cancer 1996; 73: 294-300.
24. Pei L, Melmed S, Scheithauer B, Kovacs K, Benedict WF, Prager D. Frequent loss of heterozygosity at the retinoblastoma susceptibility gene (RB) locus in aggressive pituitary tumors: evidence for a chromosome 13 tumor suppressor gene other than RB. Cancer Res 1995; 55: 1613-6.