総　説（第21回徳島医学会賞受賞論文）

Role of cyclin-dependent kinase inhibitors in pituitary tumorigenesis : an update

Md. Golam Hossain*, Takeo Iwata*, Noriko Mizusawa*, Zhi Rong Qian‡, Shozo Yamada†, Toshiaki Sano‡, and Katsuhiko Yoshimoto*

Department of *Medical Pharmacology, and ‡Department of Human Pathology, Institute of Health Biosciences, the University of Tokushima Graduate School ; and †Department of Hypothalamic and Pituitary Surgery, Toranomon Hospital, Tokyo Japan

（Received : November 21, 2008）
（Accepted : November 28, 2008）

SUMMARY

Human pituitary adenomas are common and potentially serious neoplasms that account for 10-15% of all intracranial neoplasms. In spite of extensive investigations, the molecular basis of human pituitary tumorigenesis remains elusive. The cell cycle is driven by protein complexes composed of cyclins and cyclin-dependent kinases (CDKs). CDK inhibitors (CKIs) serve as negative regulators of cell cycle. CKIs include two distinct families : the INK4 family comprising $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$ and the Cip/Kip family including $p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$. Dysregulation in CKIs are recognized as critical factors in tumorigenesis. In recent years, extensive studies have demonstrated that mutations, underexpression, and DNA methylation of the CKIs genes were frequently observed in various types of human cancers. However, the role of CKIs in human pituitary tumors has been elucidated to a limited extent. Here we review the potential role of CKIs in human pituitary adenomas concentrating on gene mutations, promoter methylation, and mRNA or protein expression levels.

Key words : pituitary adenomas, CKIs, INK4 family, Cip/Kip family, mutations, promoter methylation

Introduction

Human pituitary adenomas are the most commonly encountered intracranial neoplasms. Although pituitary adenomas are benign, it is associated with significant morbidity due to its critical location and oversecretion of pituitary hormones[1]. A large number of studies have been conducted to elaborate the molecular and pathological basis of pituitary tumorigenesis. However, the mechanisms of tumorigenesis of human pituitary adenomas are largely unknown.

The cell cycle is a tightly regulated process and is controlled at different stages by specific cyclins and cyclin-dependent kinases (CDKs). A critical point in the cell cycle is the G_1/S transition checkpoint frequently aberrated in human cancers[2,3]. CDKs are inhibited by CDK inhibitors (CKIs) which play a crucial regulatory role at G_1/S transition. To date, two families of CKIs based on their structural and functional similarities have been described. The INK4 family comprising $p16^{INK4A}$ (CDKN2A), $p15^{INK4B}$ (CDKN2B), $p18^{INK4C}$ (CDKN2C),

and $p19^{INK4D}$ (CDKN2D) shows their negative regulatory activity by binding to CDK4 and CDK6 and opposing their association with cyclin D. The Cip/Kip family including $p21^{CIP1}$ (CDKN1A), $p27^{KIP1}$ (CDKN1B), and $p57^{KIP2}$ (CDKN1C) shows a broad spectrum of inhibitory effects on cyclin/CDK complexes including cyclin D/CDK4, cyclin E/CDK2, and cyclin A/CDK2[3].

Recent studies showed that dysregulation of CKIs contributed to progression of various tumors, suggesting that CKIs act as a tumor suppressor[4, 5]. However, the role of CKIs in human pituitary tumors has been elucidated to a limited extent. We review the recent findings of CKIs associated to human pituitary tumorigenesis, with a particular focus on expression, mutations, and promoter methylation profile of CKIs. We also discuss the functional significance of collaborative roles of CKIs.

CKIs and their knock-out mice

The discovery of $p16^{Ink4a}$ and $p21^{Cip1}$ highlighted the importance of CKIs as tumor suppressors. The analysis of CKI-deficient mice further accelerated the deeper understanding of the role of CKIs in cancer research (Table 1). In human, the $p16^{INK4A}/p14^{ARF}/p15^{INK4B}$ locus on chromosome 9p21 is frequently implicated in a wide spectrum of tumors[6]. Deletion of this locus inactivates simultaneously the two members of INK4 family, $p16^{INK4A}$ and $p15^{INK4B}$, and the entirely unrelated protein, $p14^{ARF}$.

$p16^{Ink4a}$ null mice develop lymphomas and sarcomas with low penetrance[7]. The major phenotype observed in $p15^{Ink4b}$ knock-out mice was angiosarcomas with a long latency and low frequency, indicating that $p15^{Ink4b}$ has limited tumor-suppressing activities[8]. In human, the $p18^{INK4C}$ gene is located on chromosome 1p32, a region frequently altered in a variety of cancers[9]. Deletion of the $p18^{Ink4c}$ gene in mice results in the frequent development of widespread organomegaly, pituitary hyperplasia and adenomas as well as other neoplasias such as pheochromocytoma, B-cell lymphoma, angiosarcoma, thymic lymphoma, and renal cell carcinoma[8, 10]. These observations indicate that $p18^{Ink4c}$ is a tumor suppressor at least in mice. Meanwhile, deletion of the $p19^{Ink4d}$ gene does not give rise to tumors even after long observations[11]. However, mutant mice lacking both $p19^{Ink4c}$ and $p27^{Kip1}$ develop disorders such as bradykinesia, proprioceptive abnormalities, and seizures[12].

Table 1 : Major phenotypes of CKIs knock-out mice

Target	Major phenotype	References
INK4 family		
$p16^{Ink4a}$	Lymphomas and sarcomas with low penetrance	(7)
$p15^{Ink4b}$	Angiosarcomas with long latency	(8)
$p18^{Ink4c}$	Similar to phenotypes seen in mice lacking $p27^{Kip1}$	(8, 10)
$p19^{Ink4d}$	Fertility inspite of testicular atrophy	(11)
Cip/Kip family		
$p21^{Cip1}$	Histiocytic sarcomas, hemangiomas, and lymphomas	(13)
$p27^{Kip1}$	Hyperplasia in several organs and develop adenomas of intermediate lobe of the pituitary gland	(15-17)
$p57^{Kip2}$	Die immediately after birth due to dyspnea resulting from cleft palate, abdominal muscle defects or skeletal abnormalities	(18, 19)
INK4 family ; Cip/Kip family		
$p15^{Ink4b}$; $p18^{Ink4c}$	Similar to phenotypes of single knock-out mice	(8)
$p18^{Ink4c}$; $p19^{Ink4d}$	Male infertility	(54)
$p18^{Ink4c}$; $p21^{Cip1}$	Pituitary adenomas and neuroendocrine hyperplasia	(21)
$p18^{Ink4c}$; $p27^{Kip1}$	Tumors with shorter latency in endocrine glands including the pituitary	(21)
$p19^{Ink4d}$; $p27^{Kip1}$	Die of neurological defects such as bradykinesia, proprioceptive abnormalities, and seizures	(12)
CKIs ; others		
$p27^{Kip1}$; Men1	No noticeable synergistic stimulation of tumor growth	(22)
$p18^{Ink4c}$; Men1	Accelerated rate with an increased incidence of tumor development in the pituitary, thyroid, parathyroid, and pancreas	(22)
$p18^{Ink4c}$; p53	Medulloblastomas	(55)

Although the role of $p21^{Cip1}$ dificiency in the development of sarcomas and lymphomas has been defined[13], its role in pituitary tumor progression is not clear. Very recently, Chesnokova, *et al.* generated triple mutant mice ($Rb^{+/-}$; $Pttg^{-/-}$; $p21^{-/-}$) and showed that $p21^{Cip1}$ deficiency restored abrogated pituitary tumor formation in $Rb^{+/-}$; $Pttg^{-/-}$ knock-out mice, indicating the potential role of $p21^{Cip1}$ in pituitary tumor growth[14]. $p27^{Kip1}$ deficient mice display striking features of tumor development in several organs including pituitary glands[15-17]. The $p57^{Kip2}$ knock-out mice lead to developmental disor-

ders such as cleft palate and gastrointestinal abnormalities[18, 19] ; however, the role of $p57^{Kip2}$ as tumor suppressor is largely obscure. Very recently, Jin, *et al.* demonstrated that the prostates of $p57^{Kip2}$ knock-out mice developed prostatic adenocarcinomas[20].

Functional collaboration or redundancy between distinct CKIs confers higher level of regulatory roles in inhibition of tumor progression (Table 1). Although mice lacking two INK4 proteins, $p15^{Ink4b}$ and $p18^{Ink4c}$, do not develop an accelerated rate of tumors[8], but collaboration of INK4 family members with Cip/Kip family members confers decreased rate of tumor development. For example, mice lacking either $p18^{Ink4c}$ or $p27^{Kip1}$ slowly develop pituitary adenomas[8, 10, 15-17], whereas mice carrying simultaneous deletion of $p18^{Ink4c}$ and $p27^{Kip1}$ develop pituitary adenomas with more accelerated rate[21]. Generation of all four INK4 knock-out mice should be of value to better understand the INK4 family in pituitary tumor development. Interestingly, $p18^{Ink4c}$; Men1 double knock-out mice develop endocrine tumors with more accelerated rate than that of each knock-out mice[22].

The above findings denote the important role of $p18^{INK4c}$ in the development of pituitary adenomas. The analyses of double null mice clearly indicate the functional cooperation of CKIs in inhibition of pituitary tumor development (Table 1).

Mutations of the CKI genes are infrequent in pituitary tumors

Although the $p15^{INK4B}$ gene concomitant with the $p16^{INK4A}$ gene is usually deleted in a large variety of tumors[23], mutations of the $p15^{INK4B}$ gene in human tumors are infrequent[24, 25]. A more frequent mutations and deletions of the $p16^{INK4A}$ gene were reported in various malignancies[26]. However, we and others confirmed that the $p16^{INK4A}$ gene mutations are infrequent in pituitary tumors[25, 27] (Table 2). The $p18^{INK4C}$ gene mutations were rare in human cancers[28, 29]. We showed that $p18^{INK4C}$ mutations were absent in human pituitary adenomas. Very recently, van Veelen, *et al.* reported the presence of somatic inactivating missense mutations of $p18^{INK4C}$ in human medullary thyroid carcinomas and pheochromocytomas[30].

Although the majority of pituitary adenomas are sporadic, some arise as a familial syndromes. Out of CKIs, the $p27^{KIP1}$ gene is the only identified gene responsible for heritable pituitary tumors. A germline non-

Table 2 : Mutations, promoter methylation, and expression status of the CKI genes in pituitary adenomas

CKIs	Mutations	Promoter hypermethylation	Expression (mRNA or Protein)	References
INK4 family				
$p16^{INK4A}$	No mutations[25, 46], LOH (6%)[25]	52% in all subtypes and 71% of non-functioning adenomas[41-43]	Loss of protein (62%) and mRNA (93%) expression in all subtypes, especially loss of protein expression in non-functioning adenomas (79%)[42, 43, 46]	(25, 41-43, 46)
$p15^{INK4B}$	No mutations[25], LOH (6%)[25]	32-36% of adenomas[39, 40]	No reports	(25, 39, 40)
$p18^{INK4C}$	*No mutations	*5% of adenomas	Low mRNA levels in ACTH adenomas (92%) and non-functioning adenomas (83%)*[,56]	(*our results, 56)
$p19^{INK4D}$	No reports	No reports	No reports	
Cip/Kip family				
$p21^{CIP1}$	No reports	No reports	Elevated protein expression : GH (71%) and PRL adenomas (81%)[14, 49] Decreased protein expression : non-functioning adenomas (81%)[14, 49]	(14, 49)
$p27^{KIP1}$	No mutations[33, 34]	No reports	Decreased protein expression : ACTH (66%), GH (64%), PRL (56%), TSH (63%), and non-functioning adenomas (62%)[50-52]	(33, 34, 50-52)
$p57^{KIP2}$	No reports	No reports	No reports	

LOH, loss of heterozygosity ; ACTH, corticotroph ; GH, somatotroph ; PRL, lactotroph ; TSH, thyrotroph

sense mutation in the $p27^{KIP1}$ gene was identified in a multiple endocrine neoplasia type 1-suspected patient with growth hormone (GH)-secreting pituitary adenoma and parathyroid tumors[31]. An inactivating $p27^{KIP1}$ germline mutation was also deteced in a Dutch patient with hyperparathyroidism, a corticotroph (ACTH) pituitary adenoma, and a neuroendocrine carcinoid tumor[32]. These results confirmed the potential role of $p27^{KIP1}$ in genesis of pituitary adenomas. However, several studies revealed infrequent mutations of $p27^{KIP1}$ in sporadic pituitary adenomas[33,34], suggesting that mutations of CKIs play a limited role in human pituitary tumorigenesis.

Promoter methylation of the CKI genes in pituitary adenomas

Epigenetic inactivation by promoter methylation is one of the important mechanisms of gene silencing in human cancers[35]. Promoter methylation implicated in abberant gene expression is a hallmark of human pituitary tumorigenesis[36]. Hypermethylation-associated down-regulated mRNA expression of the $p15^{INK4B}$ gene appears to be a common event in human lymphoid tumors and mouse T-cell lymphomas[37,38].

Ogino, *et al.* reported that the $p15^{INK4B}$ gene promoter was hypermethylated in 36% of pituitary adenomas[39]. But they did not demonstrate the effect of promoter hypermethylation on $p15^{INK4B}$ expression. These results warrant further investigations to establish the correlation between protein or mRNA expression of $p15^{INK4B}$ and promoter hypermethylation of the gene in pituitary adenomas. Promoter hypermethylation is a common mechanism of $p16^{INK4A}$ inactivation in various tumors including pituitary adenomas[39-43] (Table 2). Methylation-associated silencing of the $p16^{INK4A}$ gene is more frequent in non-functioning pituitary adenomas than other subtypes and is associated with loss of $p16^{INK4A}$ protein expression[41-43]. These suggest subtype-specific deregulation of the $p16^{INK4A}$ gene in pituitary tumors. It is considered that epigenetic inactivation by methylation is an early event of pituitary tumorigenesis[42]. In contrast, Seemann, *et al.* demonstrated that loss of $p16^{INK4A}$ expression and its promoter methylation were related to larger tumor size, suggesting that $p16^{INK4A}$ deregulation is achieved during adenoma progression rather than an early event[44]. We showed reduced expression of $p18^{INK4C}$ in both mRNA and protein levels, but absence of promoter hypermethylation in human pituitary adenomas.

Although $p27^{Kip1}$ expression was reported to be down-regulated in pituitary adenomas, promoter hypermethylation as well as methylation-associated down-regulation of Cip/Kip family members were not reported. Collectively, these data indicate that pituitary adenomas have epigenetic changes in $p16^{INK4A}$ of CKIs.

Deregulated expression of CKIs in pituitary adenomas

$p15^{INK4B}$ expression is shown to be down-regulated in mitogen-stimulated lymphocytes[45]. However, the levels of $p15^{INK4B}$ expression in pituitary adenomas remain elusive. Loss of $p16^{INK4A}$ expression is frequent in non-functioning adenomas[42,46] (Table 2). In non-functioning pituitary adenomas, we showed reduced mRNA expression of $p18^{INK4C}$. Ramsey, *et al.* showed a compensatory role between $p16^{Ink4a}$ and $p18^{Ink4c}$ in mice[47], but we did not observe their compensatory expression in human pituitary adenomas.

$p21^{CIP1}$ deletion or mutation is not a common feature in human tumors[48]. Interestingly, tumor growth triggers elevated expression of $p21^{CIP1}$ in GH adenomas[14,49]. Deregulated expression of $p27^{KIP1}$ is frequent in a wide variety of human malignancies and is considered as a prognostic marker for clinical outcome of human cancers[4]. Down-regulated expression of $p27^{KIP1}$ does not result from inactivating mutations of the $p27^{KIP1}$ gene in pituitary as well as other tumors. $p27^{KIP1}$ protein levels are down-regulated by other mechanisms such as proteolytic degradation, cytoplasmic mislocalization etc[4]. Several studies showed that $p27^{KIP1}$ protein expression is down-regulated in pituitary adenomas[50-52] (Table 2). The lower levels of $p27^{KIP1}$ protein in ACTH adenomas are of particular interest, because intermediate lobe-derived pituitary tumors developed in $p27^{Kip1}$ knock-out mice[15-17]. In ACTH tumors, an accentuated phosphorylation of $p27^{KIP1}$ leading to its increased degradation is

observed[53]. These results indicate that phosphorylation of $p27^{KIP1}$ may play a role in pituitary tumorigenesis.

Concluding remarks

Each of the INK4 and Cip/Kip family members shows unique pattern of mutations, gene expression, and promoter methylation. However, the collaborative properties suggest diverse roles for the individual CKIs in regulation of pituitary tumorigenesis.

References

1. Heaney, A. P., Melmed, S. : Molecular targets in pituitary tumours. Nat. Rev. Cancer, **4** : 285-295, 2004
2. Ortega, S., Malumbres, M., Barbacid, M. : Cyclin-D dependent kinases, INK4 inhibitors and cancer. Biochim. Biophys. Acta, **1602** : 73-87, 2002
3. Grana, X., Reddy, E. P. : Cell cycle control in mammalian cells : role of cyclins, cyclin-dependent kinases (CDKs), growth suppressor genes and cyclin-dependent kinase inhibitors (CKIs). Oncogene, **11** : 211-219, 1995
4. Besson, A., Dowdy, S. F., Roberts, J. M. : CDK inhibitors : cell cycle regulators and beyond. Dev. Cell, **14** : 159-169, 2008
5. Thullberg, M., Bartkova, J., Khan, S., Hansen, K., *et al.* : Distinct versus redundant properties among members of the INK4 family of cyclin-dependent kinase inhibitors. FEBS Letters, **470** : 161-166, 2000
6. Gil, J., Peters, G. : Regulation of the INK4b-ARF-INK 4a tumor suppressor locus all for one or one for all. Nat. Rev. Mol. Cell Biol., **7** : 667-677, 2006
7. Sharpless, N. E., Bardeesy, N., Lee, K. H., Carrasco, D., *et al.* : Loss of $p16^{Ink4a}$ with retention of $p19^{Arf}$ predisposes mice to tumorigenesis. Nature, **413** : 86-91, 2001
8. Látres, E., Malumbres, M., Sotillo, R., Martín, J., *et al.* : Limited overlapping roles of $p15^{INK4b}$ and $p18^{INK4c}$ cell cycle inhibitors in proliferation and tumorigenesis. EMBO J., **19** : 3496-3506, 2000
9. Guan, K. L., Jenkins, C. W., Li, Y., Nichols, M. A., *et al.* : Growth suppression by p18, a p16INK4/MTS1- and p14INK4B/MTS2-related CDK6 inhibitor, correlates with wild-type pRb function. Genes Dev., **8** : 2939-2952, 1994
10. Franklin, D. S., Godfrey, V. L., Lee, H., Kovalev, G. I., *et al.* : CDK inhibitors $p18^{INK4c}$ and $p27^{Kip1}$ mediate two separate pathways to collaboratively suppress pituitary tumorigenesis. Genes Dev., **12** : 2899-2911, 1998
11. Zindy, F., van Deursen, J., Grosveld, G., Sherr, C. J., *et al.* : INK4d-deficient mice are fertile despite testicular atrophy. Mol. Cell. Biol., **20** : 372-278, 2000
12. Zindy, F., Cunningham, J. J., Sherr, C. J., Jogal, S., *et al.* : Postnatal neuronal proliferation in mice lacking Ink4d and Kip1 inhibitors of cyclin-dependent kinases. Proc. Natl. Acad. Sci. USA, **96** : 13462-13467, 1999
13. Martín-Caballero, J., Flores, J. M., García-Palencia, P., Serrano, M. : Tumor susceptibility of $p21^{Waf1/Cip1}$-deficient mice. Cancer Res., **61** : 6234-6238, 2001
14. Chesnokova, V., Zonis, S., Kovacs, K., Ben-Sholomo, A., *et al.* : $p21^{Cip1}$ restrains pituitary tumor growth. Proc. Natl. Acad. Sci. USA, **105** : 17498-17503, 2008
15. Fero, M. L., Rivkin, M., Tasch, M., Porter, P., *et al.* : A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility $p27^{Kip1}$-deficient mice. Cell, **85** : 733-744, 1996
16. Nakayama, K., Ishida, N., Shirane, M., Inomata, A., *et al.* : Mice lacking $p27^{Kip1}$ display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. Cell, **85** : 707-720, 1996
17. Kiyokawa, H., Kineman, R. D., Manova-Todorova, K. O., Soares, V. C., *et al.* : Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of $p27^{Kip1}$. Cell, **85** : 721-732, 1996
18. Yan, Y., Frisen, J., Lee, M. H., Massague, J., *et al.* : Ablation of the CDK inhibitor $p57^{Kip2}$ results in increased apoptosis and delayed differentiation during mouse development. Genes Dev., **11** : 973-983, 1997
19. Zhang, P., Liegeois, N. J., Wong, C., Finegold, M. : Altered cell differentiation and proliferation in mice lacking $p57^{Kip2}$ indicates a role in Beckwith-Wiedemann syndrome. Nature, **387** : 151-158, 1997
20. Jin, R. J., Lho, Y., Wang, Y., Ao, M., *et al.* : Down-

regulation of $p57^{Kip2}$ induces prostate cancer in the mouse. Cancer Res., 68：3601-3608, 2008

21. Franklin, D. S., Godfrey, V. L., O'Brien, D. A., Deng, C., *et al.* : Functional collaboration between different cyclin-dependent kinase inhibitors suppresses tumor growth with distinct tissue specificity. Mol. Cell. Biol., 20：6147-6158, 2000
22. Bai, F., Pei, X. H., Nishikawa, T., Smith, M. D., *et al.* : $p18^{Ink4c}$, but not $p27^{Kip1}$, collaborates with Men1 to suppress neuroendocrine organ tumors. Mol. Cell. Biol., 27：1495-1504, 2007
23. Stone, S., Dayananth, P., Jiang, P., Weaver-Feldhaus, J. M., *et al.* : Genomic structure, expression and mutational analysis of the p15 (MTS2) gene. Oncogene, 11：987-991, 1995
24. Lin, Y. W., Chen, C. H., Huang, G. T., Lee, P. H., *et al.* : Infrequent mutations and no methylation of CDKN 2A (p16/MTS1) and CDKN2B (p15/MTS2) in hepatocellular carcinoma in Taiwan. Eur. J. Cancer, 34：1789-1795, 1998
25. Yoshimoto, K., Tanaka, C., Yamada, S., Kimura, T., *et al.* : Infrequent mutations of $p16^{INK4a}$ and $p15^{INK4b}$ genes in human pituitary adenomas. Eur. J. Endocrinol., 136：74-80, 1997
26. Ruas, M., Peters, G. : The $p16^{INK4a}$/CDKN2A tumor suppressor and its relatives. Biochim. Biophys. Acta, 1378：115-177, 1998
27. Jaffrain-Rea, M. L., Ferretti, E., Toniato, E., Cannita, K., *et al.* : p16 (INK4a, MTS-1) gene polymorphism and methylation status in human pituitary tumors. Clin. Endocrinol. (Oxf), 51：317-325, 1999
28. Böstrom, J., Meyer-Puttlitz, B., Wolter, M., Blaschke, B., *et al.* : Alterations of the tumor suppressor genes CDKN2A ($p16^{INK4a}$), $p14^{ARF}$, CDKN2B ($p15^{INK4b}$), and CDKN2C ($p18^{INK4c}$) in atypical and anaplastic meningiomas. Am. J. Pathol., 159：661-669, 2001
29. Otsuki, T., Jaffe, E. S., Wellmann, A., Kumar, S., *et al.* : Absence of p18 mutations or deletions in lymphoid malignancies. Leukemia, 10：356-360, 1996
30. van Veelen, W., Klompmaker, R., Gloerich, M., van Gastteren, C. J. R., *et al.* : P18 is a tumor suppressor gene involved in human medullary thyroid carcinoma and pheochromocytoma development. Int. J. Cancer, 124：339-345, 2009
31. Pellegata, N. S., Quintanilla-Martinez, L., Siggelkow, H., Samson, E., *et al.* : Germ-line mutations in $p27^{Kip1}$ cause a multiple endocrine neoplasia syndrome in rats and humans. Proc. Natl. Acad. Sci. USA, 103：15558-15563, 2006
32. Georgitsi, M., Raitila, A., Karhu, A., van der Luijt, R. B., *et al.* : Germline CDKN1B/$p27^{Kip1}$ mutation in multiple endocrine neoplasia. J. Clin. Endocrinol. Metab., 92：3321-3325, 2007
33. Takeuchi, S., Koeffler, H. P., Hinton, D. R., Miyoshi, I., *et al.* : Mutation and expression analysis of the cyclin-dependent kinase inhibitor gene p27/Kip1 in pituitary tumors. J. Endocrinol., 157：337-341, 1998
34. Tanaka, C., Yoshimoto, K., Yang, P., Kimura, T., *et al.* : Infrequent mutations of $p27^{Kip1}$ gene and trisomy 12 in a subset of human pituitary adenomas. J. Clin. Endocrinol. Metab., 82：3141-3147, 1997
35. Esteller, M. : Epigenetic gene silencing in cancer : the DNA hypermethylome. Hum. Mol. Genet., 16：R50-R59, 2007
36. Farrell, W. E., Clayton, R. N. : Epigenetic change in pituitary tumorigenesis. Endocr. Relat. Cancer, 10：323-330, 2003
37. Herman, J. G., Jen, J., Merlo, A., Baylin, S. B. : Hypermethylation-associated inactivation indicates a tumor suppressor role for $p15^{INK4b}$. Cancer Res., 56：722-727, 1996
38. Malumbres, M., Perez de Castro, I., Santos, J., Melendez, B., *et al.* : Inactivation of the cyclin-dependent kinase inhibitor $p15^{INK4b}$ by deletion and *de novo* methylation with independence of $p16^{INK4a}$ alterations in murine primary T-cell lymphomas. Oncogene, 14：1361-1370, 1997
39. Ogino, A., Yoshino, A., Katayama, Y., Watanabe, T., *et al.* : The $p15^{INK4B}$/$p16^{INK4A}$/RB1 pathway is frequently deregulated in human pituitary adenomas. J. Neuropathol. Exp. Neurol., 64：398-403, 2005
40. Yoshino, A., Katayama, Y., Ogino, A., Watanabe, T., *et al.* : Promoter hypermethylation profile of cell cycle regulator genes in pituitary adenomas. J. Neurooncol., 83：153-162, 2007
41. Woloschak, M., Yu, A., Post, K. D. : Frequent inacti-

vation of the $p16^{INK4a}$ gene in human pituitary tumors by gene methylation. Mol. Carcinog., **19**：221-224, 1997

42. Simpson, D. J., Bicknell, J. E., McNicol, A. M., Calyton, R.: Hypermethylation of the p16/CDKN2A/MTS1 gene and loss of protein expression is associated with nonfunctional pituitary adenomas but not somatotrophinomas. Genes Chromosomes Cancer, **24**：328-336, 1999
43. Ruebel, K. H., Jin, L., Zhang, S., Scheithauer, B. W., *et al.*: Inactivation of the p16 gene in human pituitary nonfunctioning tumors by hypermethylation is more common in null cell adenomas. Endocr. Pathol., **12**：281-289, 2001
44. Seemann, N., Kuhn, D., Wrocklage, C., Keyvani, K., *et al.*: CDKN2A/p16 inactivation is related to pituitary adenoma type and size. J. Pathol., **193**：491-497, 2001
45. Lois, A. F., Cooper, L. T., Geng, Y., Nobori, T., *et al.*: Expression of the p16 and p15 cyclin-dependent kinase inhibitors in lymphocyte activation and neuronal differentiation. Cancer Res., **55**：4010-4013, 1995
46. Woloschak, M., Yu, A., Xiao, J., Post, K. D.: Frequent loss of the $p16^{INK4a}$ gene product in human pituitary tumors. Cancer Res., **56**：2493-2496, 1996
47. Ramsey, M. R., Krishnamurthy, J., Pei, X. H., Torrice, C., *et al.*: Expression of $p16^{INK4a}$ compensates for $p18^{INK4c}$ loss in cyclin-dependent kinase 4/6-dependent tumors and tissues. Cancer Res., **67**：4732-4741, 2007
48. Shiohara, M., El-Deiry, W. S., Wada, M., Nakamaki, T.: Absence of WAF1 mutations in a variety of human malignancies. Blood, **84**：3781-3784, 1994
49. Neto, A. G., McCutcheon, I. E., Vang, R., Spencer, M. L., *et al.*: Elevated expression of p21（WAF1/Cip1）in hormonally active pituitary adenomas. Ann. Diagn. Pathol., **9**：6-10, 2005
50. Komatsubara, K., Tahara, S., Umeoka, K., Sanno, N., *et al.*: Immunohistochemical analysis of p27（Kip1）in human pituitary glands and in various types of pituitary adenomas. Endocr. Pathol., **12**：181-188, 2001
51. Bamberger, C. M., Fehn, M., Bamberger, A. M., Lüdecke, D. K., *et al.*: Reduced expression levels of the cell-cycle inhibitor $p27^{Kip1}$ in human pituitary adenomas. Eur. J. Endocrinol., **140**：250-255, 1999
52. Lidhar, K., Korbontis, M., Jordan, S., Khalimova, Z., *et al.*: Low expression of the cell cycle inhibitor $p27^{Kip1}$ in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors. J. Clin. Endocrinol. Metab., **84**：3823-3830, 1999
53. Korbonits, M., Chahal, H. S., Kaltsas, G., Jordan, S., *et al.*: Expression of phosphorylated $p27^{Kip1}$ protein and jun activation domain binding protein 1 in human pituitary adenomas. J. Clin. Endocrinol. Metab., **87**：2635-2643, 2001
54. Zindy, F., den Besten, W., Chen, B., Rehg, J. E., *et al.*: Control of spermatogenesis in mice by the cyclin D-dependent kinase inhibitors $p18^{Ink4c}$ and $p19^{Ink4d}$. Mol. Cell. Biol., **21**：3244-3255, 2001
55. Uziel, T., Zindy, F., Xie, S., Lee, Y., *et al.*: The tumor suppressors Ink4c and p53 collaborate independently with patched to suppress medulloblastoma formation. Genes Dev., **19**：2656-2667, 2005
56. Morris, D. G., Musat, M., Czirják, S., Hanzély, Z., *et al.*: Differential gene expression in pituitary adenomas by oligonucleotide array analysis. Eur. J. Endocrinol., **153**：143-151, 2005