Analysis of Cyclin D1 (*CCND1*) Allelic Imbalance and Overexpression in Sporadic Human Pituitary Tumors[1]

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ABSTRACT

Cyclin D1 plays an important role in the regulation of cell progression through G_1 of the cell cycle and has been demonstrated to have oncogenic properties. Using RFLP-PCR, an A/G polymorphism within the cyclin D1 (*CCND1*) gene was analyzed in 151 sporadic human pituitary tumors, of which 60 were informative at this locus. Further analysis showed that in 15 of 60 (25%) tumors, there was evidence of allelic imbalance, which is indicative of gene amplification. Allelic imbalance was observed more frequently in invasive tumors (11 of 29 tumors; 38%) than in their noninvasive counterparts (4 of 31 tumors; 13%; $P = 0.02$). Forty-six of the tumors informative for the polymorphism were available for immunohistochemical analysis. Cyclin D1 expression (nuclear and/or cytoplasmic) was detected in 25 of 46 (54%) tumors. Of these cases, expression of nuclear cyclin D1 was detected in 9 of 46 (20%) tumors, whereas 16 of 46 (35%) tumors showed cyclin D1 staining exclusively confined to the cytoplasm. Neither nuclear staining nor cytoplasmic staining was observed in any of the normal pituitaries or in the negative control. Expression of cyclin D1 was observed in significantly more nonfunctional tumors (18 of 27 tumors; 67%) than in somatotrophinomas (7 of 19 tumors; 37%; $P = 0.046$). Nuclear cyclin D1 expression was observed more frequently in nonfunctional tumors (8 of 27 tumors; 30%) than in somatotrophinomas (1 of 19 tumors; 5%; $P = 0.04$). There was no correlation between cyclin D1 expression and tumor grade or between allelic imbalance of *CCND1* and cyclin D1 expression. We conclude that amplification of *CCND1* occurs in pituitary tumors and that the overexpression of cyclin D1 may be an early event in tumorigenesis. Cyclin D1 overexpression occurring in the absence of *CCND1* allelic imbalance suggests that additional mechanisms responsible for deregulated cyclin D1 expression are involved in human pituitary tumorigenesis.

INTRODUCTION

The deregulation of genes involved in the control of the cell cycle is one of the most common alterations in tumor growth; cells with such a defect often have a growth advantage over their neighbors. Progression through G_1 to the S phase of the cycle is mediated by the interplay between proteins controlling pRb[3] phosphorylation. Under mitogenic stimulation, the cyclin D1 protein binds to cyclin-dependent kinase 4, which is subsequently activated and phosphorylates pRb. In a hyperphosphorylated form, pRb is inactivated, and the cells are released from G_1 arrest. In contrast, under growth-inhibitory conditions, kinase inhibitors such as the p16 protein bind competitively to cyclin-dependent kinase 4, effectively keeping the cell in G_1 and thus preventing cell division (1–3).

The role of cyclin D1 in the regulation of transition through G_1 into S phase has been demonstrated *in vitro*. Cyclin D1 overexpression has been shown to shorten the duration of G_1, whereas in studies using cyclin D1 antibodies or antisense plasmid, passage of the cell into the S phase of the cell cycle is prevented (4, 5). The oncogenic nature of the cyclin D1 gene was shown by its introduction into murine 3T3 cells, causing their transformation (6). Overexpression of cyclin D1 in transgenic mice leads to tumor formation in the mammary glands (7) and the lymphatic system (8). The cyclin D1 gene (*CCND1*), located at 11q13, is one of the most frequently amplified genes observed in human tumors, with amplification of *CCND1* frequently leading to cyclin D1 protein overexpression (1–3). In head and neck cancers (9, 10), cyclin D1 amplification and overexpression is associated with a poor prognosis, whereas in other cancers such as breast (11), bladder (12), and non-small cell lung cancers (13), overexpression of cyclin D1 correlates with good prognosis. In tumors, overexpression of cyclin D1 may also arise from chromosomal translocations, such as that observed in parathyroid adenomas (14) and lymphomas (15).

Although cyclin D1 has been studied extensively in a number of human malignancies, including breast, lung, and bladder cancers, lymphomas, and ovarian tumors, cyclin D1 expression has not been examined in pituitary tumors. Human pituitary tumors are responsible for an estimated 10% of all

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[3] The abbreviations used are: pRb, retinoblastoma protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

intracranial tumors. Although the majority of these tumors will remain benign, approximately one-third become invasive, and a small number become malignant. Such tumors are believed to arise as a result of an accumulation of genetic aberrations, including the silencing of tumor suppresser genes and/or the activation of oncogenes (16). With the exception of the pituitary tumor-transforming gene (17) and the G protein oncogene *Gsp* (reviewed in Ref. 18), little is known about the role of oncogenes in pituitary tumorigenesis. Previous studies by our group and others have demonstrated that the *p16* gene is frequently methylated and that protein expression is commonly lost in pituitary tumors (19, 20). We aimed to determine whether cyclin D1, another member of the pathway that controls the progression through G_1 into S phase, is deregulated in pituitary tumorigenesis. This study analyzes *CCND1* amplification, as assessed by allelic imbalance, and investigates the expression and subcellular location of cyclin D1 using immunohistochemistry in normal and neoplastic pituitaries.

MATERIALS AND METHODS

Patient and Tumor Material. The total tumor cohort ($n = 156$) comprised 60 somatotrophinomas and 96 nonfunctional tumors, together with patient-matched blood samples. The tumors were defined as invasive or noninvasive as a result of clinical evaluation (computed tomography and/or magnetic resonance imaging scans) and graded according to a modified Hardy (21) classification as described in a previous report (22). 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 grade 1 and 2 tumors were considered noninvasive. Grade 3 tumors were locally invasive with evidence of bony destruction and tumor within the sphenoid and/or cavernous sinus. Grade 4 tumors demonstrate central nervous system/extracranial spread with or without metastases. Both grade 3 and 4 tumors were considered to be invasive. Based on these criteria, the nonfunctional tumors comprised 45 noninvasive and 51 invasive tumors. The somatotrophinomas cohort comprised 40 noninvasive tumors and 20 invasive tumors. Table 1 details the grade of the individual tumors used in this study. In addition to tumor material, six normal pituitaries were obtained within 12 h postmortem and served as controls.

RFLP-PCR Analysis of *CCND1*. To analyze the allelic status of the cyclin D1 gene (*CCND1*), we have exploited the single-bp polymorphism within the splice donor region of exon 4 that creates a restriction site that is cleaved by the restriction enzyme *Scr*FI (23). Using PCR, blood leukocyte DNA from individuals ($n = 156$) with pituitary tumors was analyzed for *Scr*FI heterozygosity. The allele frequencies and genotype distribution for this polymorphism in patients with pituitary tumors are reported elsewhere.[4] The corresponding tumors were analyzed for allelic imbalance in those cases informative for the polymorphism.

Tumor and leukocyte DNA was obtained using standard methods (Nucleon DNA isolation kit; Anachem, Bedfordshire, United Kingdom) and subjected to PCR amplification using specific *CCND1* oligonucleotides (sense, GGGACATCAC-CCTCACTTAC; antisense, GCAGTGCAAGGCCTGAACCT) designed to flank the *Scr*FI polymorphism. As an internal control, the housekeeping gene *GAPDH* (sense, CCCTTTTGTAG-GAGGGACT; antisense, AATGCTTGCTGCTGCCTA) was coamplified in each reaction. Primers for the *GAPDH* gene were designed to produce an amplicon, which did not contain the *Scr*FI restriction site. All samples were analyzed at least twice. PCR reactions were carried out in 25-μl volumes with 1.5 mM $MgCl_2$; 200 μM each of dATP, dGTP, dTTP, and dCTP; 2 pmol of each primer template DNA; and 1 unit of Taq DNA polymerase. PCR amplification occurred with an annealing temperature of 60°C with the addition of 3.6% formamide to the PCR buffer. PCR was limited to 28–30 cycles to ensure amplification in the linear range.

The PCR product (5 μl) was digested with 1 unit of *Scr*FI restriction enzyme at 37°C for 3 h. Digested products (AA genotype, 113 bp; GG genotype, 91 and 22 bp; AG genotype, 113, 91, and 22 bp) were visualized on 8% nondenaturing polyacrylamide gels, fixed in 10% methylated spirit and 0.5% acetic acid for 6 min, and then incubated in 0.1% aqueous silver nitrate for 15 min. After two brief washes in distilled water, products were visualized by development in 1.5% sodium hydroxide and 0.1% formaldehyde.

Assessment of Allelic Imbalance. DNA samples that were heterozygous after digestion were assessed for allelic imbalance of *CCND1* by comparing the allele intensity in the tumor with that in the matched leukocyte DNA. Tumors were scored positive for allelic imbalance if the intensity of one of the alleles in the tumor DNA showed a more intense staining relative to the alleles present in the matched blood DNA sample (23). As an additional control, imbalance was only assigned after the relative intensity of the coamplified GAPDH amplicon was taken into account.

Immunohistochemistry. Of the total cohort studied, 60 cases were informative for the *CCND1* polymorphism, and sufficient material was available for immunohistochemical analysis in 46 cases. The immunohistochemistry used in this study was a modification of the method described by Rosenberg *et al.* (14) and was performed using a Shandon sequenza (Shandon). Briefly, formalin-fixed paraffin-embedded sections were air-dried on 3-aminopropyltriethoxysilane (2%, v/v)-coated slides. After dewaxing, the sections were treated with hydrogen peroxide (3%, v/v) in methanol to block endogenous peroxidase activity and washed in PBS. Sections were subjected to microwave antigen retrieval in 10 mM citrate buffer (pH 6.0) for 15 min. Immunohistochemistry was performed using Vectastain Universal Elite ABC kit (Vector Laboratories). Cyclin D1 was detected using a mouse monoclonal antibody (DCS-6; Novocastra), used at a dilution of 1:100 and left on sections for 30 min. Detection was performed using diaminobenzene (Sigma) for 5 min. Sections were then counterstained with hematoxylin, dehydrated, and mounted. A positive control (human tonsil), a negative control (pituitary tumor with the primary antibody omitted), and normal human pituitaries were used for each series of staining. The resulting sections were examined blind, without

[4] D. J. Simpson, P. R. Hoban, N. A. Hibberts, A. A. Fryer, R. N. Clayton, and W. E. Farrell. Cyclin D1 (*CCND1*) genotype defines tumor grade in pituitary adenoma, submitted for publication.

Table 1 Analysis of cyclin D1 expression in pituitary tumors together with clinical diagnosis and tumor grade

Pituitary no.	Clinical diagnosis[a]	Tumor grade	Nuclear staining	Cytoplasmic staining	Allelic imbalance
76	Inv NF	3	−	++	No
145	Inv NF	3	−	++	No
147	Inv NF	3	+	+	No
151	Inv NF	3	−	++	No
222	Inv NF	3	+	−	Yes
224	Inv NF	3	−	++	No
236	Inv NF	3	−	++	No
237	Inv NF	3	−	−	Yes
245	Inv NF	3	−	+	Yes
259	Inv NF	4	++	++	Yes
261	Inv NF	3	−	−	No
366	Inv NF	3	+	+	No
388	Inv NF	3	−	++	No
8	Non-Inv NF	2	−	−	No
12	Non-Inv NF	2	−	++	No
13	Non-Inv NF	2	−	−	No
95	Non-Inv NF	2	+	−	No
100	Non-Inv NF	2	−	−	No
137	Non-Inv NF	2	+	+	No
138	Non-Inv NF	2	−	−	No
154	Non-Inv NF	2	−	++	No
156	Non-Inv NF	2	−	++	No
157	Non-Inv NF	2	−	−	No
205	Non-Inv NF	2	+	+	No
218	Non-Inv NF	2	−	−	No
389	Non-Inv NF	2	−	−	No
393	Non-Inv NF	2	+	−	No
3	Inv Somato	3	−	−	No
18	Inv Somato	3	−	−	No
133	Inv Somato	3	−	−	Yes
164	Inv Somato	3	−	−	Yes
171	Inv Somato	3	−	−	No
240	Inv Somato	4	−	++	Yes
348	Inv Somato	3	++	−	Yes
363	Inv Somato	3	−	−	Yes
72	Non-Inv Somato	1	−	++	No
122	Non-Inv Somato	1	−	−	No
149	Non-Inv Somato	2	−	−	No
162	Non-Inv Somato	2	−	+	No
167	Non-Inv Somato	2	−	−	No
168	Non-Inv Somato	2	−	−	Yes
170	Non-Inv Somato	2	−	−	Yes
173	Non-Inv Somato	2	−	+	No
217	Non-Inv Somato	2	−	++	No
265	Non-Inv Somato	2	−	−	No
398	Non-Inv Somato	2	−	+++	No

[a] Inv NF, invasive nonfunctional tumor; Non-Inv NF, noninvasive nonfunctional tumor; Inv Somato, invasive somatotrophinoma; Non-Inv Somato, noninvasive somatotrophinoma.

any prior knowledge of pituitary subtype or grade, and classified as follows, according to standard protocols: (*a*) −, 0–10% of cells stained positive; (*b*) +, 10–50% of cells stained positive; (*c*) ++, 50–70% of cells stained positive; and (*d*) +++, >70% of cells stained positive. Nuclear staining was defined as those sections showing nuclear staining alone or nuclear and cytoplasmic staining. Cytoplasmic staining was defined as those sections showing cytoplasmic staining alone.

Statistical Analysis. Statistical analysis was carried out using the Stat-Calc program (Epi Info Version 5.01; Public Domain Software). Fischer's exact test was used to analyze any differences between the cyclin D1 staining patterns and tumor types and to analyze the relationship between *CCND1* allelic imbalance and tumor type or grade. χ^2 analysis was used to study any correlation between allelic imbalance and cyclin D1 immunostaining.

RESULTS

Analysis of *CCND1* Polymorphism and Allelic Imbalance. A total of 60 of 156 (38.5%) patient blood leukocyte DNA samples amplified with the cyclin D1 primers were heterozygous for the *Scr*FI polymorphism. This was not significantly different from the frequency of 49% found in a control population by Betticher *et al.* (23). These paired blood/tumor samples were then assessed for allelic imbalance within the

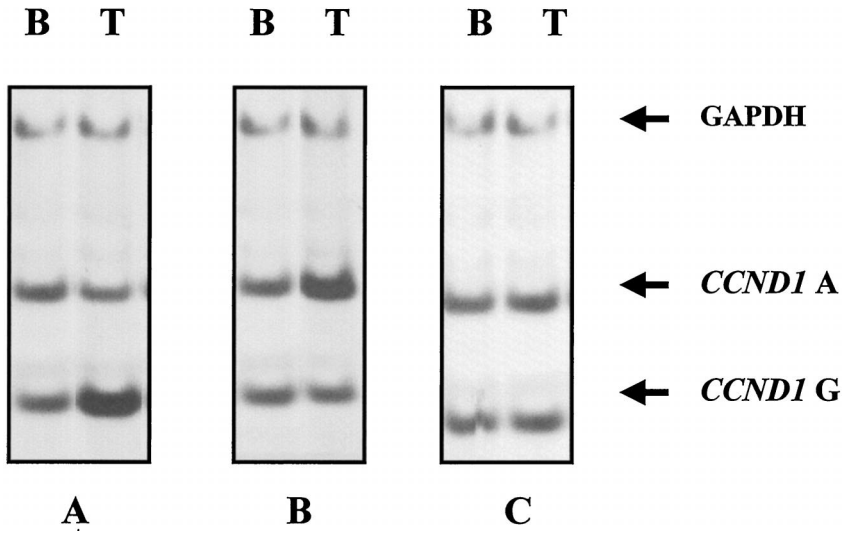

Fig. 1 Allelic imbalance of the *CCND1* gene in human pituitary tumors. *A* shows allelic imbalance as a more intense G allele in the tumor (*T*) relative to the matched blood (*B*) sample. *B* shows allelic imbalance of the A allele, whereas in *C*, no evidence of allelic imbalance is present. The 22-bp fragment is not shown in the figures.

CCND1 gene. Fig. 1 shows a representation of allelic imbalance of the *CCND1* gene in human pituitary tumors. Fig. 1*A* shows allelic imbalance as a more intense G allele relative to the matched blood sample. Fig. 1*B* shows allelic imbalance of the A allele, whereas in Fig. 1*C*, no evidence of allelic imbalance is present. In tumors examined for imbalance, there was no bias toward a specific allele (A or G) being amplified. Imbalance of *CCND1* was observed in 15 of 60 (25%) pituitary tumors. Imbalance was detected more frequently in invasive tumors (11 of 29 tumors; 38%) than in noninvasive tumors (4 of 31 tumors; 14%; $P = 0.02$). There were no statistically significant differences in the allelic imbalance observed between nonfunctional tumors (6 of 34 tumors; 18%) and somatotrophinomas (9 of 26 tumors; 35%). Table 1 details the status of allelic imbalance of the *CCND1* gene in individual pituitary tumors.

Immunohistochemical Analysis of Cyclin D1 Expression and Subcellular Location. Having demonstrated *CCND1* allelic imbalance in 25% of tumors, we were interested to establish whether this was associated with overexpression of the cyclin D1 protein. Of those tumors informative for the *Scr*FI polymorphism, 46 were available for immunohistochemical analysis. Expression of nuclear and/or cytoplasmic cyclin D1 was observed in 25 of 46 (54%) pituitary tumors studied. This staining pattern was variable, and slides were graded as described previously (Table 1). Expression of cyclin D1 was observed in the nucleus of 9 of 46 (20%) tumors, whereas staining was observed exclusively in the cytoplasm in 16 of 46 (35%) tumors. In the normal pituitaries ($n = 6$), no staining for cyclin D1 was observed in the nucleus or cytoplasm (Table 2). Representative photographs of nuclear and cytoplasmic staining are shown in Fig. 2, together with the normal pituitary.

Association between Cyclin D1 Staining and Tumor Characteristics. Expression of nuclear and/or cytoplasmic cyclin D1 was observed in significantly more of the nonfunctional tumors (18 of 27 tumors; 67%) than somatotrophinomas (7 of 19 tumors; 37%; $P = 0.046$). Nuclear staining was observed significantly more frequently in nonfunctional tumors (8 of 27 tumors; 30%) than in somatotrophinomas (1 of 19 tumors; 5%; $P = 0.04$). There was no significant difference in the cytoplasmic cyclin D1 staining between nonfunctional tumors (10 of 27 tumors; 37%) and somatotrophinomas (6 of 19 tumors; 32%). There was no significant correlation between nuclear or cytoplasmic cyclin D1 expression and the grade of tumor studied, with noninvasive tumors showing expression of cyclin D1 in 12 of 25 (48%) tumors, compared to 13 of 21 (62%) invasive tumors (Table 2).

Association between Allelic Imbalance and Cyclin D1 Staining. In this study, 60 paired blood/tumor samples were investigated for allelic imbalance of *CCND1*. Sufficient material was available to allow 46 of these tumors to be analyzed for both CCND1 amplification and cyclin D1 expression, with 11 tumors showing *CCND1* amplification. A total of 5 of these 11 tumors (45%) showed both *CCND1* amplification (as assessed by allelic imbalance) and cyclin D1 overexpression, whereas 6 of 11 tumors (55%) showed *CCND1* allelic imbalance that was not accompanied by expression of cyclin D1. No correlation was observed between the overexpression of cyclin D1 and *CCND1* allelic imbalance. Table 1 summarizes the data and shows the expression of cyclin D1 in tumors with and without evidence of imbalance at the *CCND1* locus.

DISCUSSION

It is well documented that the D-type cyclins are involved in the regulation of the G_1–S-phase transition of the cell cycle. Overexpression of cyclin D1 has been shown to shorten the G_1 phase of the cell cycle and lead to tumor formation (reviewed in Refs. 3 and 24). The mechanism responsible for deregulated cyclin D1 expression has been investigated in many human tumors, with gene amplification being commonly reported. Whereas gene amplification can be defined by Southern blot analysis, other recent reports have detailed this phenomenon by allelic imbalance studies within the *CCND1* gene (13, 23). Using this technique, we were able to analyze a large cohort of pituitary tumors in which the DNA was obtained from microdissected archival material. Allelic imbalance was observed in

Table 2 Immunohistochemical analysis of cyclin D1 expression in normal pituitary and pituitary tumors

Nuclear staining was defined as those sections showing nuclear staining alone or nuclear and cytoplasmic staining, whereas cytoplasmic staining was defined as those sections showing cytoplasmic staining alone.

	Nuclear staining	Cytoplasmic staining
Normal pituitary ($n = 6$)	0/6 (0%)	0/6 (0%)
Total pituitary tumor cohort ($n = 46$)	9/46 (20%)	16/46 (35%)
Invasive pituitary tumors ($n = 21$)	5/21 (24%)	8/21 (38%)
Noninvasive pituitary tumors ($n = 25$)	4/25 (16%)	8/25 (32%)
Nonfunctional tumors ($n = 27$)	8/27 (30%)	10/27 (37%)
Somatotrophinomas ($n = 19$)	1/19 (5%)	6/19 (32%)

this study in 15 of the 60 informative pituitary tumors examined, with imbalance observed more frequently in invasive tumors (11 of 29 tumors) than in their noninvasive counterparts (4 of 31 tumors). There was no correlation between the overexpression of cyclin D1 and *CCND1* allelic amplification. Although imbalance of the *CCND1* gene is usually associated with overexpression of cyclin D1, there have been several studies showing amplification without overexpression (reviewed in Ref. 12). The amplification of *CCND1* without cyclin D1 overexpression seen in this study might be explained by coamplification of the 11q13 chromosomal region containing other potential oncogenes such as *EMS1* (reviewed in Ref. 25). Interestingly, while this study was in progress, Metzger *et al.* (26) found 11q13 rearrangement in 4 of 52 pituitary tumors using comparative genomic hybridization. Immunohistochemical staining for cyclin D1 was investigated; however, none of the tumors with 11q13 rearrangement showed cyclin D1 positivity.

Other mechanisms that have been implicated in the inappropriate expression of cyclin D1 include cytogenetic rearrangements of the *CCND1* gene. For example, in some parathyroid tumors, an inversion of 11q13 and 11q15 causes amplification of *CCND1* (14), whereas in lymphomas, a chromosomal translocation at the *bcl-1* breakpoint causes *CCND1* to be up-regulated because it comes under the control of the immunoglobulin heavy chain enhancer (15). However, no evidence for rearrangement at the *bcl-1* locus has been found in pituitary tumors (27).

In this study, we report the overexpression of cyclin D1 in pituitary tumors. Positive immunohistochemical staining (in which at least 10% of neoplastic cells were stained) was observed in 54% of tumors, compared to a complete absence of staining in the normal pituitary. Nuclear staining for cyclin D1 was evident in 9 of 46 tumors, whereas exclusively cytoplasmic staining was observed in 16 of 46 tumors. The overexpression of cyclin D1 has been reported in many human malignancies including breast, ovarian, and head and neck cancers and lymphomas, with both nuclear and cytoplasmic cyclin D1 staining being well-recognized events (11, 15, 28–30). Interestingly, Lukas *et al.* (31) showed that in the U-2-OS cell line, cytoplasmic staining for cyclin D1 occurred during the G_1-S transitional phase of the cell cycle, with nuclear staining visualized only in G_1. The authors suggested a differential solubility of the cyclin D1 protein to explain the apparent cell cycle stage subcellular localization. The importance of cytoplasmic cyclin D1 expression in tumors showing this phenomenon is not currently understood.

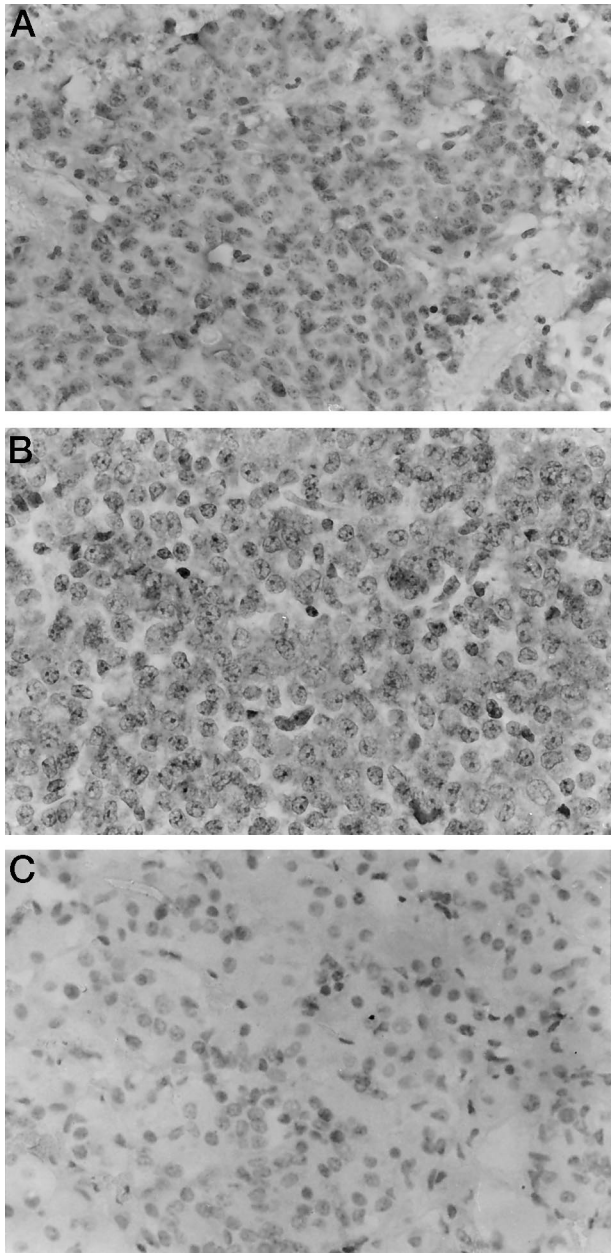

Fig. 2 Immunohistochemical analysis of cyclin D1 in primary human pituitary tumors. *A*, positive immunostaining is seen in the nuclei of cells, accompanied by a lower level of cytoplasmic staining. *B*, staining for cyclin D1 is seen only in the cytoplasm of cells. *C*, no staining for cyclin D1 is seen in the normal pituitary. Magnification, ×200.

Expression of nuclear and/or cytoplasmic cyclin D1 was observed in significantly more of the nonfunctional tumors

(67%) than somatotrophinomas (37%). Nuclear staining for cyclin D1 was observed in significantly more nonfunctional tumors than in somatotrophinomas (30% *versus* 5%, respectively), indicating that the inappropriate expression of cyclin D1 may play a more important role in nonfunctional pituitary tumors than somatotrophinomas. This finding is in agreement with previous studies that have shown that the expression of different genes or proteins is altered during the tumorigenesis of different pituitary subtypes. Thus, the *Gsp* oncogene, which results in a constitutively active G protein, is most commonly observed in somatotrophinomas (reviewed in Ref. 18), whereas the *p16* gene is more frequently methylated in nonfunctional tumors (20).

In our study, the expression of cyclin D1 (nuclear and/or cytoplasmic) did not correlate with the biological behavior of the pituitary tumor, with 12 of 25 noninvasive tumors showing expression of cyclin D1, compared to 13 of 21 invasive tumors. These findings suggest that overexpression of cyclin D1 is an early event in pituitary tumorigenesis. Previous studies have also shown that in other human tumors, such as colorectal (32), ovarian (33), lung (34), and breast (35) tumors, cyclin D1 expression is an early event in tumorigenesis and is maintained in more advanced stages of the disease.

The expression of proteins such as cyclin D1, pRb, and p16, which are involved in regulation of the G_1-S-phase transition, is frequently altered in human tumors (reviewed in Ref. 24). This study provides further evidence that the deregulation of genes involved in the G_1-S-phase transition is important in pituitary tumorigenesis. In addition to the deregulation of cyclin D1, recent studies (19, 20) have demonstrated that p16 protein expression is lost in a high percentage of pituitary tumors. The generally accepted view is that deregulation of pRb, p16, and cyclin D1 appears to be mutually exclusive (reviewed in Ref. 1); however, studies in some human cell lines (36) and in primary lung (33) and breast (37) cancer have shown cases where deregulation of p16, pRb, and cyclin D1 occurred simultaneously. It would therefore be interesting to examine the possibility that such cooperation occurs in pituitary tumorigenesis.

In conclusion, this study shows that allelic imbalance of *CCND1* was detected by RFLP-PCR in human pituitary tumors, with imbalance observed more frequently in invasive tumors than in their noninvasive counterparts. Cyclin D1 protein was frequently overexpressed in human pituitary tumors compared to the normal pituitary, where no staining was observed. Nuclear staining was observed in significantly more nonfunctional tumors than somatotrophinomas. No difference in cyclin D1 expression was observed between noninvasive and invasive tumors, which suggests that the expression of cyclin D1 may be deregulated early in pituitary tumorigenesis. Whereas there was no correlation between allelic imbalance and protein overexpression, we cannot exclude this as a mechanism in a proportion of tumors overexpressing cyclin D1. However, in tumors expressing protein without an allelic imbalance, our study suggests that other mechanisms are responsible for the overexpression of cyclin D1 in pituitary tumorigenesis.

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