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The Expression of G1-S Cell Cycle Inhibitors in Normal Placenta and Gestational Trophoblastic Diseases

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Background : The objective of this study is to determine the expression pattern of G1-S inhibitor molecules in normal trophoblasts and gestational trophoblastic diseases, including hydatidiform moles and choriocarcinoma. **Methods :** A total of 157 cases comprising 47 normal placentas and 110 gestational trophoblastic diseases such as choriocarcinoma (19 cases) and hydatidiform moles (91 cases of which 58 were complete, 12 were partial and 21 were invasive mole) were immunohistochemically analyzed on paraffin blocks using anti-p21, anti-p27, anti-p16, anti-p53, anti-pRb antibodies. **Results :** The results revealed that in the normal placenta, all the G1-S cell cycle inhibitors were maximally expressed by the first-trimester trophoblasts and these levels decreased with gestational age. The expression of p21 and p53 was greatly enhanced in the gestational trophoblastic diseases, particularly in invasive mole and choriocarcinoma, whereas the p27 expression was significantly downregulated in choriocarcinoma. Especially, Rb expression was typically enhanced in the invasive mole, but not in choriocarcinoma. The expression level of p16 was low in all the cases, and particularly in choriocarcinoma. **Conclusions :** In conclusion, we demonstrated that the expression of G1/S cell cycle inhibitors correlates well with normal trophoblast differentiation, and these expressions are considerably altered in the gestational trophoblastic diseases, including complete/partial/invasive hydatidiform mole and choriocarcinoma.

Key Words : Cell Cycle Inhibitors; Trophoblast; Hydatidiform Mole; Choriocarcinoma; Immunohistochemistry

Dysregulated cell proliferation is the hallmark of cancer, and tumor cells endure damage to the genes that directly regulate their cell cycles. In the cell cycle, the period from the late G1 to the S phase is the most important period for cell proliferation.[1] This phase functions as an intricate system of safeguards and checkpoints that are mainly mediated by p53 and pRb, and this facilitates quality control by constantly surveying the ordered progression through the cell division cycle. Phosphorylation of pRb occurs via a molecular on/off switch at the G1/S checkpoint and this is induced by cyclin D1-cyclin-dependent kinase (cdk) 4 or the cdk6 complex, and the cyclin E-cdk2 complex.[1] A number of cdk inhibitors (CKIs) that bind with specific cdks prevent unrestrained proliferation. The two CKI subtypes include the INK (inhibitor of kinase) family, which includes p16 (INK4a), p15 (INK4b), p18 (INK4c) and p19 (INK4d), and the cdk-interacting protein/kinase-inhibitory protein (CIP/KIP) family, which includes p21 (WAF1/CIP1), p27 (KIP1) and p57 (KIP2).[2] It has been demonstrated that the p21 expression is regulated by wild-type p53. However, the p53-independent pathways that result in p21 induction may represent an alternative pathway to control cell growth.[3]

The expression of the major G1/S inhibitors, including p53, pRb, p16, p21 and p27, in the placenta is relatively unknown.[4-7] In addition, there have been no comprehensive studies regard-

ing the expression of major G1/S inhibitors in the various placental tissues of the normal placenta at each trimester and of the various gestational trophoblastic diseases such as complete/partial/invasive mole and choriocarcinoma.

In this study, we conducted a comprehensive, systematic analysis of the expression of G1-S inhibitors at the protein level by using immunohistochemistry methods in cases that involved a normal placenta or gestational trophoblastic disease. The results revealed a significant alteration in the expression of G1-S inhibitors in these gestational trophoblastic diseases.

MATERIALS AND METHODS

Patients, tissue samples and reagents

We investigated 157 patients and they included those cases with a normal placenta and those cases that had a gestational trophoblastic disease. The data on the abovementioned cases was obtained from the surgical pathology files maintained at the Department of Pathology of Chungbuk National University Hospital and Samsung Medical Center. The selected cases comprised 19 cases of choriocarcinoma, 91 cases of hydatidiform moles (58 complete, 12 partial and 21 invasive moles), and 47 cases with a normal placenta (17 first trimester, 10 s trimester and 20 third trimester). The diagnosis of hydatidiform mole was conducted based on the histologic findings and patient history, and p57 immunostaining was done in some indefinite cases. The gestational age of the moles ranged from 5 to 17 weeks and the mean gestational age was 9.97 weeks. Most molar cases occurred during the first trimester and the early second trimester. Permission for this study was granted from the institutional review board of Chungbuk National University Hospital and Samsung Medical Center.

Tissue microarray slides enabled successful detection of G1-S inhibitors. To prepare these slides, tissue columns (3.0 mm in diameter) were punched from the original blocks and they were inserted into new paraffin blocks (each containing 30 holes to accommodate the tissue columns); consequently, serially sectioned slides were produced. Each microarray tissue slide (1 × 3 inch) retained 30 specimens, and this allowed simultaneous analysis of 30 specimens with only minimum discrepancy in the staining process. All the specimens were circular in shape with a 3.0-mm diameter; this provided us with a sufficient amount of tissue surface for histopathologic analysis.

All the archival materials were routinely fixed in 10% neutral-buffered formalin and then they were embedded in paraffin. Four micrometer sections were prepared on silane-coated slides (Sigma, St Louis, MO, USA). The immunostaining kits were purchased from DAKO Inc. (Glostrup, Denmark).

Immunohistochemical staining

The tissue sections on the microslides were deparaffinized with xylene, hydrated in serial dilutions of alcohol and then immersed in 3% H_2O_2 to quench the endogenous peroxidase activity. For antigen retrieval, the sections were then microwaved for 20 min in 10-mM citrate buffer (pH 6.0) and 40-mM borate buffer (pH 8.2) supplemented with 1-mM EDTA and 1-mM NaCl.[8,9] This was followed by incubation with various primary antibodies (anti-p27, anti-p21, anti-p16, anti-p53 and anti-pRb). The dilution ratio and optimal retrieval buffer for each antibody are listed in Table 1. Primary antibody incubation was carried out for 60 min, followed by 3 successive rinses with a washing buffer. Further incubation was performed using a dextran polymer conjugated with peroxidase and rabbit anti-mouse Ab (DAKO, Envision plus, Carpinteria, CA, USA) for an additional 20 min at room temperature. After rinsing, the slides were wash- ed and the chromogen was developed for 5 min with liquid 3,3'-diaminobenzidine (DiNonA, Seoul, Korea). The slides were counter stained with Meyer's hematoxylin, dehydrated and then mounted with Canada balsam for examination. We used distilled water with 0.1% Tween 20 as a rinsing solution.[10]

Evaluation of the results of immunohistochemical staining

In this study, we employed the scoring method described by Sinicrope *et al*.[11] for evaluating the intensity of immunohisto-

Table 1. The antibodies and retrieval buffers for each antibody

	Nature of antibody	Company	Catalogue number	Dilution ratio	Retrieval buffer
P27	Mouse monoclonal	Santa-Cruz	sc-7966	1:50	Borate buffer
P21	Mouse monoclonal	Neomarker	MS-387-P1	1:80	Borate buffer
P16	Mouse monoclonal	Dako Cytomation	K5334	1:30	Citrate buffer
P53	Mouse monoclonal	Novocastra Laboratories Ltd	NCL-p53-DO7	1:50	Borate buffer
RB	Mouse monoclonal	Novocastra Laboratories Ltd	NCL-RB	1:50	Borate buffer

RB, retinoblastoma.

chemical staining and the proportion of stained epithelial cells. The staining intensity was subclassified as follows: 1) weak, 2) moderate or 3) strong. The positive cells were quantified as a percentage of the total number of epithelial cells and the results were assigned to one of the five categories (0: <5%, 1: 5-25%, 2: 26-50%, 3: 51-75% and 4: >75%). In order to generate the IHC (immunohistochemistry) score for each of the tumor specimens, the percentage of the positive stained tumor cells counted on the slide and the staining intensities were multiplied. Each lesion was separately examined and then scored by two pathologists (X.Y.H & S.H.K). Those cases with a discrepancy in the scores were discussed in order to reach a consensus.

Statistical analysis

Statistical analyses were conducted using Fisher's exact tests, Pearson's χ^2 tests, ANOVA, Mann-Whitney tests, Tukey's HSD test and Duncan's test (as a *post hoc* test). The differences were considered statistically significant at a p value less than 0.05. All statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA).

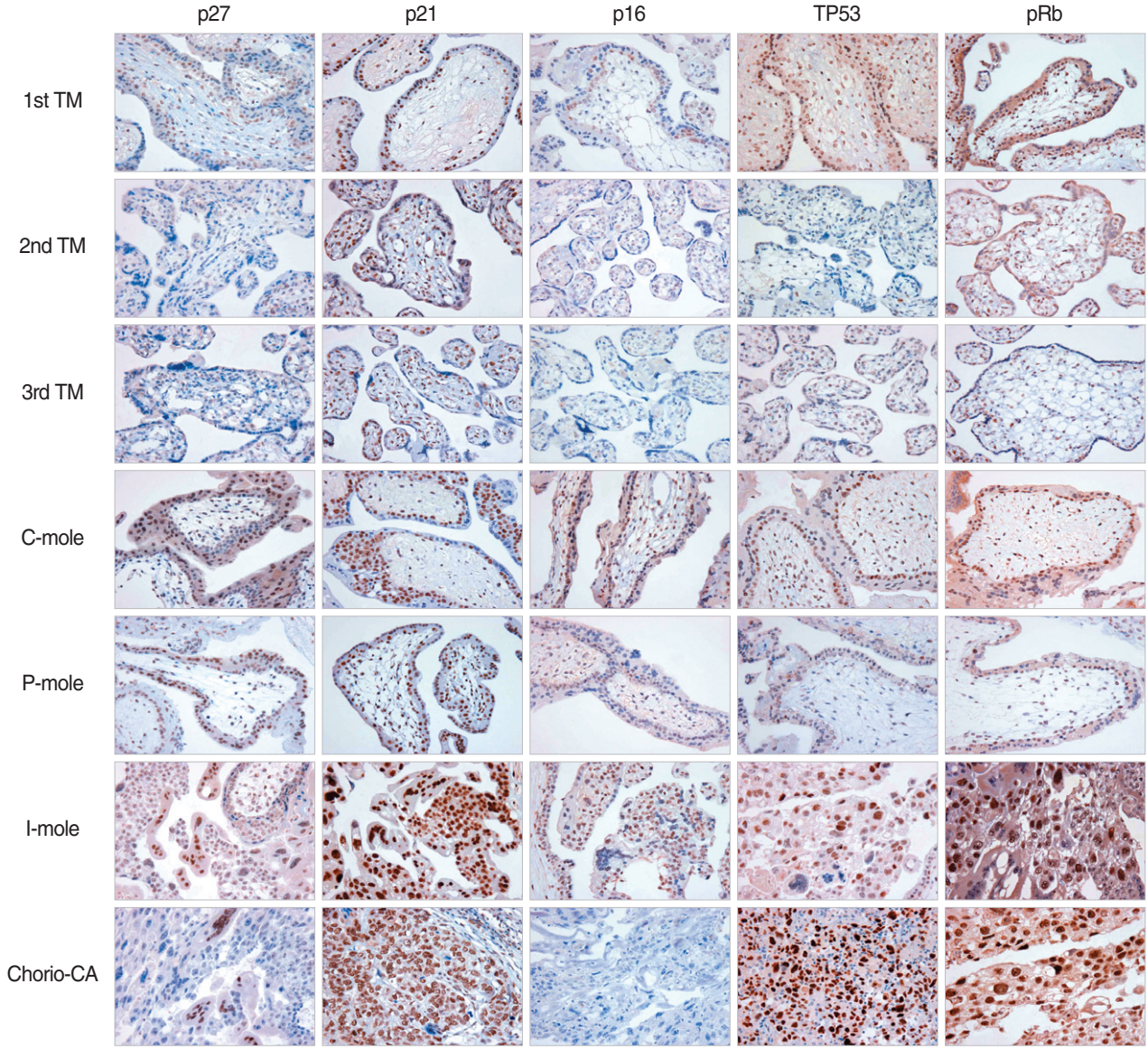


Fig. 1. The immunostaining of G1/S signaling molecules in the normal placenta and premalignant and malignant placental tumors. 1st TM, first-trimester placenta; 2nd TM, second-trimester placenta; 3rd TM, third-trimester placenta; C-mole, complete mole; P-mole, partial mole; I-mole, invasive mole; Chorio-CA, choriocarcinoma.

RESULTS

The expressional patterns of the G1-S cell cycle inhibitors in the normal placenta and the various types of gestational trophoblastic diseases, including choriocarcinoma and invasive mole/partial mole/complete mole, are depicted in Table 2 and Fig. 1, 2. The average immunostaining intensity (the mean of the IHC scores) was also analyzed.

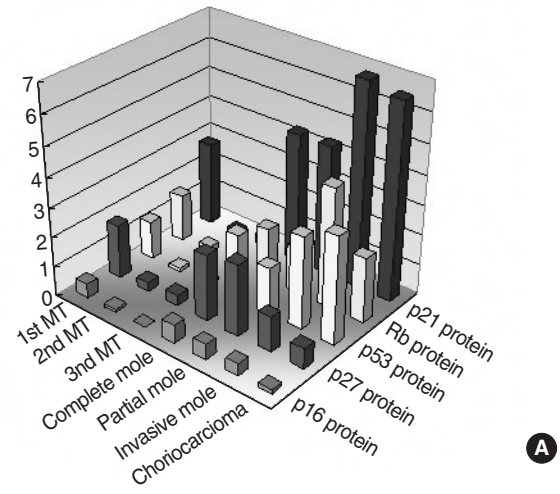
TP53 expression

The TP53 levels varied in the chorionic villous trophoblasts of the normal placenta, depending on the trimesters. Specifically, this expression was relatively weak in the first trimester (IHC score: 1.29±1.05) and very low in the second (IHC score: 0.10 ±0.10) and third trimesters (IHC score: 0.20±0.10) (Table 2, Fig. 2). In the first trimester, the TP53 expression was very weak in the nucleus of the syncytiotrophoblasts and it was at slightly higher levels in the cytotrophoblasts (Fig. 1). Except in the case of partial mole, the TP53 expression in all the gestational trophoblastic diseases was generally upregulated. The protein level in the complete mole cases (IHC score: 2.22±0.98) was higher than that observed for any trimester of the normal placenta ($p<0.025$) (Table 2, Fig. 2). The TP53 expression levels in the case of the invasive mole and choriocarcinoma were higher (IHC score: 3.11±1.32, 3.61±3.36, respectively) than those in the complete and invasive moles (complete mole vs invasive mole/choriocarcinoma; $p<0.001$) (Table 2, Fig. 2).

Table 2. The mean IHC scores of G1/S cell cycle inhibitors in the gestational trophoblastic diseases and normal placenta

Diagnosis	p27		p21		p16		p53		RB	
1st trimester placenta	1.82±1.01	p<0.001	2.82±2.19	p<0.001	0.47±0.62	NS	1.29±1.05	p<0.001	1.59±1.28	p<0.001
2nd trimester placenta	0.30±0.48		0.10±0.32		0.10±0.32		0.10±0.32		0.20±0.42	
3rd trimester placenta	0.37±0.50		0.35±1.14		0±0.22		0.10±0.31		0.25±0.44	
Complete mole	2.23±1.28		4.36±2.29		0.61±1.00		2.22±0.98		1.75±1.63	
Partial mole	2.40±1.07		4.36±2.58		0.50±0.80		1.58±1.08		1.55±0.82	
Invasive mole	1.19±1.40		6.83±3.09		0.42±1.02		3.11±1.32		4.05±2.25	
Choriocarcinoma	0.68±1.00		6.59±2.00		0.14±0.36		3.61±3.36		2.19±1.83	

IHC score, immunohistochemistry score; NS, not significant; RB, retinoblastoma.



A

Diagnosis	p16	p27	p53	pRb	p21
1st TM	±	±	±	+	++
2nd TM	-	-	-	-	-
3nd TM	-	-	-	-	-
C-Mole	±	+	+	+	+++
P-Mole	±	+	+	+	+++
I-Mole	±	±	++	+++	++++
Chorio-CA	-	±	+++	+	++++

p27, 1st TM vs 2nd & 3rd TM: $p<0.001$. 1st TM vs choriocarcinoma: $p<0.01$. Complete/partial mole vs incasive mole & choriocarcinoma: $p<0.001$ & $p<0.01$.
p21, 1st TM vs 2nd & 3rd TM: $p<0.001$. 1st TM vs Complete/invasive mole & choriocarcinoma: $p<0.05$ & $p<0.001$ & $p<0.001$. Complete mole vs invasive mole & choriocarcinoma: $p<0.001$. Partial mole vs invasive mole & choriocarcinoma: $p<0.01$.
p53, 1st TM vs 2nd & 3rd TM: $p<0.05$. 1st TM vs Complete/invasive mole & choriocarcinoma: $p<0.05$ & $p<0.001$ & $p<0.001$. Complete mole vs invasive mole & choriocarcinoma: $p<0.05$ & $p<0.001$. Partial mole vs invasive mole & choriocarcinoma: $p<0.01$ & $p<0.001$.
pRb, 1st TM vs 2nd & 3rd TM: $p<0.05$ & $p<0.01$. 1st TM vs invasive mole: $p<0.001$. Complete/partial mole & choriocarcinoma vs invasive mole: $p<0.001$.

B

Fig. 2. The expression profile of each G1/S cell cycle inhibitor in the tissue samples obtained from the normal placenta and its related premalignant and malignant lesions. (A) The bar chart illustrates the mean IHC score of each inhibitor in each normal placenta and placental lesion. (B) A summary of the results . Criteria; IHC score of 0-0.4, -; 0.4-1.5, ±; 1.5-2.5, +; 2.5-3.5, ++; 3.5-4.5, +++; >4.5, ++++. 1st TM, first trimester placenta; 2nd TM, second trimester placenta; 3rd TM, third trimester placenta.

p21 expression

The p21 expression pattern observed in the chorionic villous trophoblasts of the normal placenta was similar to that of TP53, i.e., it varied according to the gestational age. As noted earlier, the p21 expression was relatively weak in the first trimester (IHC score: 2.82±2.19) and it was very weak in the second (IHC score: 0.10±0.10) and third trimesters (IHC score: 0.20 ±0.35) (Table 2, Fig. 1, 2). In the first trimester, the p21 expression was relatively weak in the cytotrophoblasts (Fig. 1). The expression of p21 in the gestational trophoblastic diseases was also analogous to that observed for TP53. Specifically, the p21 expression was clearly increased in trophoblastic diseases as compared to that for any trimester of the normal trophoblasts. Moreover, the p21 expression in the case of the invasive mole and choriocarcinoma (IHC score: 6.83±3.09/6.59±2.00, respectively) was higher than that in the complete and partial moles (IHC score: 4.36±2.29/4.36±2.58, respectively), with a statistical significance of $p<0.01$.

p16 expression

The expression of p16 was generally weak and relatively rare in the normal trophoblasts belonging to various trimesters, as well as in the gestational trophoblastic diseases (Table 2, Fig. 1, 2). In the first-trimester chorionic villi, the p16 expression was

Table 3. Immunoreactivity of G1/S inhibitors in tissues obtained from the normal placenta and trophoblastic disease placenta

	1st TM		2nd TM		3rd TM		CM	PM	IM	CA
	STB	CTB	STB	CTB	STB	CTB				
Genbacev *et al.* (Genbacev *et al.*, 2000)										
p16	−	−	++	++	−	±				
p21	−	+	−	±	±	±				
p27	−	+	−	±	+	±				
p53 (P)	−	−	−	−	−	−				
Quenby *et al.* (Quenby *et al.*, 1998)										
p21	±	+++								
p53	−	±								
Rb	++	++								
Bamberger *et al.* (Bamberger *et al.*, 1999)										
p27	++	−	++	+	++	+				
Olvera *et al.* (Olvera *et al.*, 2001)										
p27	−	+++	−	+	−	±	++	+++		±
Marzusch *et al.* (Marzusch *et al.*, 1995)										
p53	−	+	−	+	−	+				
Fulop *et al.* (Fulop *et al.*, 1998)										
p53			Normal trophoblast: ±				+++	±		+++
p21			Normal trophoblast: +				+++	++		+++
Rb			Normal trophoblast: +++				+++	+++		+++
Cheung *et al.* (Cheung *et al.*, 1999)										
p53			Normal trophoblast: 2.6%				29.9%	14.1%		30.7%
Kale *et al.* (Kale *et al.*, 2001)										
p53			Normal trophoblast: 7.8%				71.2%	24.5%	78.3%	
Halperin *et al.* (Halperin *et al.*, 2000)										
p53			Normal trophoblast: 3.0%				15.0%	8.0%		
Yang *et al.* (Yang *et al.*, 2002)										
p53			Normal trophoblast: 7.8 %				54.9%		89.5%	88.9%
Lee *et al.* (Lee, 1995)										
p53			STB: (−), CTB: weak postive				100%	82.0%		88.0%
Cheville *et al.* (Cheville *et al.*, 1996)										
p53			Normal trophoblast: 8.9%				41.0%	28.0%		
Al-Bozom *et al.* (Al-Bozom, 2000)										
p53			Normal trophoblast: 0.0%				93.0%	57.0%		
Cheung *et al.* (Cheung *et al.*, 1998)										
p21			Normal trophoblast: 5.2%				20.7%	9.8%		23.3%

p53(P), phosphorylated p53 protein.
STB, syncytiotrophoblast; CTB, cytotrophoblast; CM, complete mole; PM, partial mole; IM, invasive mole; CA, choriocarcinoma.

restricted to the nucleus of the first-trimester cytotrophoblasts (Fig. 1). The villous trophoblasts of various moles displayed a weak p16 nuclear expression (Fig. 1). In the choriocarcinomas, a p16 expression was only detected in 2 of the 14 cases (14.3%) (Tables 2, 3). The level of the p16 expression in the complete mole cases was significantly higher than that in the third-trimester trophoblasts ($p<0.01$). However, in the other cases, the p16 expression level did not appear to be significantly different from that in the normal placenta and the gestational trophoblastic diseases (Table 2, Fig. 2).

p27 expression

In the normal placenta, the p27 expression was restricted to the nucleus of the first-trimester villous trophoblasts (IHC score: 1.82 ± 1.02), and it was very low for the second (IHC score: 0.30 ± 0.48) and third trimesters (IHC score: 0.37 ± 0.50), with a significant difference of p 0.001 (Table 2, Fig. 2). For the first trimester, the p27 expression was mainly restricted to the cytotrophoblasts (Fig. 1).

In the gestational trophoblastic diseases, moderate p27 expression levels were noted in the complete and partial mole cases (IHC score: $2.23 \pm 1.28/2.40 \pm 1.07$, respectively), which was similar to that observed for the first-trimester trophoblasts (Table 2, Fig. 2). However, in the cases of the invasive mole and choriocarcinoma, the p27 expression was typically reduced and their levels (IHC score: $1.19 \pm 1.40/0.68 \pm 1.00$, respectively) were significantly lower than those in the complete and partial moles and the first-trimester trophoblasts ($p<0.01$) (Table 2, Fig. 2).

pRb expression

The pRb expression levels in the chorionic villous trophoblasts of the normal placenta also varied depending on the trimesters. Specifically, the pRb expression was also relatively limited to the first trimester (IHC score: 1.59 ± 1.28), and it was infrequent and very weak in the second (IHC score: 0.20 ± 0.42) and third trimesters (IHC score: 0.25 ± 0.44) with a significant difference of $p<0.025$ (Table 2, Fig. 2). In the first trimester, the pRb expression was also generally limited to the cytotrophoblasts and not to the syncytiotrophoblasts (Fig. 1).

In gestational trophoblastic diseases, except for the invasive mole, the pRb expression levels were generally similar to those in the first-trimester normal trophoblasts. However, the level in the case of the invasive moles (IHC score: 4.05 ± 2.25) was much higher than that in all the other gestational trophoblastic diseases (complete mole/partial mole/choriocarcinoma, IHC score: $1.75 \pm 1.63/1.55 \pm 0.82/2.19 \pm 1.83$, respectively) and the normal placenta ($p<0.001$) (Table 2, Fig. 2). Similar to the other abovementioned proteins, pRb was mainly expressed in the nucleus, although a cytoplasmic expression was also observed in certain cases.

DISCUSSION

In this study, we determined the expression pattern of G1/S cell-cycle inhibitors in the normal trophoblasts and gestational trophoblastic diseases, including the complete mole/partial mole/invasive mole and choriocarcinoma. Our results demonstrated extensive and dynamic changes in the expression profiles of the G1/S cell cycle inhibitors, as were revealed in the spectrum of these lesions. The first issue to discuss is the functional implication of these characteristic expressional patterns of G1/S inhibitors.

Gestational trophoblastic diseases are characterized by abnormally high levels of trophoblast proliferation.[12,13] Therefore, there may be a very strong mitotic drive that is accompanied by strong counter-activation of G1/S inhibitors as a result of a feedback mechanism. Choriocarcinoma is a highly malignant epithelial tumor that may arise from the trophoblasts of any type of gestational event, and most commonly in the case of a complete mole. In choriocarcinoma, certain G1/S inhibitors, including p21, TP53 and pRb, were paradoxically overexpressed, whereas p27 and p16 were downregulated.

The paradoxical overexpression of tumor suppressor genes has been reported in a variety of human cancers such as those of the esophagus, lung, breast and colon.[14] Further, an expression pattern of G1/S inhibitors that was highly similar to that obtained in this study, including the pattern for the over-expression of p21 and TP53 and the concurrent down-regulation of p21, has also been reported in the gynecological cancers such as ovarian[15] and uterine cervical cancer.[16] The paradoxical increase in the tumor suppressor protein expression suggests the existence of a feedback loop between the oncogenes and the tumor suppressor genes; its purpose is to maintain a homeostatic balance between the positive and negative regulators of the G_1-S transition in the cell cycle.[14] From this viewpoint, p27 and p16 downregulation in choriocarcinoma is indicative of a disrupted feedback mechanism; this may partly account for the uncontrolled proliferation of the transformed trophoblasts. In the case of p16, its expres-

sion level was very low in the normal trophoblasts and gestational trophoblastic diseases, suggesting there was mutation or promoter hypermethylation of the *p16* gene.

Overall, our results are consistent with those of the previous studies (Table 3). It was reported that TP53 was upregulated in gestational trophoblastic diseases compared to that in the normal placental trophoblasts, and this is in spite of minor differences regarding the details in the other studies.[17-23] Regarding the normal placental trophoblasts, some studies have revealed that TP53 was mainly expressed in the cytotrophoblasts and not in the syncytiotrophoblasts.[7,24] With respect to p27, previous research has reported conflicting results on the immunoreactivity of p27 in the normal trophoblasts.[5,6,25] However, our results are highly consistent with those reported by Olvera *et al*.[6] It has also been reported that p21 displays immunoreactivity in the normal trophoblasts, and particularly in the first-trimester cytotrophoblasts,[4,5,7,26] and p21 shows an increased expression in gestational trophoblastic diseases.[4,26] Overall, these results were consistent with those obtained from our study. Quenby *et al*. and Fulop *et al*. reported that pRb was expressed in the normal trophoblasts,[4,7] and the pRB expression was high in complete moles and choriocarcinoma.[4] These results are generally consistent with ours, but our paper has reported for the first time the typically high pRB expression in invasive moles. Genbacev *et al*. reported the expression of p16 in the normal trophoblasts.[5] They discovered strong immunoreactivity of p16 in both syncytiotrophoblasts and cytotrophoblasts of the second trimester and no or very weak immunoreactivity in the trophoblasts of the first and third trimesters.[5] This is different from our findings, which revealed that a p16 expression was restricted to the first-trimester trophoblasts. This discrepancy may have resulted from the practical differences between immunofluorescent staining and immunohistochemistry. However, the results reported by Genbacev *et al*.[5] appear to be inconsistent with the overall expressional pattern of G1/S cell cycle inhibitors, which tend to be downregulated in the second and third trimester-trophoblasts.

In summary, we have demonstrated that the expression of G1/S cell cycle inhibitors is greatly altered in gestational trophoblastic diseases, including complete/partial/invasive hydatidiform moles and choriocarcinoma. Specifically, the enhanced expression of p21, p53 and Rb in gestational trophoblastis disease and the reduced expression of p27 in choriocarcinoma suggests that p21, p21, p53 and Rb and the G1-S cell cycle inhibitors may be implicated in the pathogenesis of these diseases.

REFERENCES

1. Schafer KA. The cell cycle: a review. Vet Pathol. 1998; 35: 461-78.
2. Sherr CJ. Cancer cell cycles. Science 1996; 274: 1672-7.
3. Steinman RA, Hoffman B, Iro A, Guillouf C, Liebermann DA, el-Houseini ME. Induction of p21 (WAF-1/CIP1) during differentiation. Oncogene 1994; 9: 3389-96.
4. Fulop V, Mok SC, Genest DR, Gati I, Doszpod J, Berkowitz RS. p53, p21, Rb and mdm2 oncoproteins. Expression in normal placenta, partial and complete mole, and choriocarcinoma. J Reprod Med 1998; 43: 119-27.
5. Genbacev O, McMaster MT, Fisher SJ. A repertoire of cell cycle regulators whose expression is coordinated with human cytotrophoblast differentiation. Am J Pathol 2000; 157: 1337-51.
6. Olvera M, Harris S, Amezcua CA, *et al*. Immunohistochemical expression of cell cycle proteins E2F-1, Cdk-2, Cyclin E, p27 (kip1), and Ki-67 in normal placenta and gestational trophoblastic disease. Mod Pathol 2001; 14: 1036-42.
7. Quenby S, Brazeau C, Drakeley A, Lewis-Jones DI, Vince G. Oncogene and tumour suppressor gene products during trophoblast differentiation in the first trimester. Mol Hum Reprod 1998; 4: 477-81.
8. Kim SH, Kook MC, Shin YK, Park SH, Song HG. Evaluation of antigen retrieval buffer systems. J Mol Histol 2004; 35: 409-16.
9. Kim SH, Kook MC, Song HG. Optimal conditions for the retrieval of CD4 and CD8 antigens in formalin-fixed, paraffin-embedded tissues. J Mol Histol 2004; 35: 403-8.
10. Kim SH, Shin YK, Lee KM, Lee JS, Yun JH, Lee SM. An improved protocol of biotinylated tyramine-based immunohistochemistry minimizing nonspecific background staining. J Histochem Cytochem 2003; 51: 129-32.
11. Sinicrope FA, Ruan SB, Cleary KR, Stephens LC, Lee JJ, Levin B. bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res 1995; 55: 237-41.
12. Fox H. Aging of the placenta. Arch Dis Child Fetal Neonatal. Ed 77, 1997; 171-5.
13. Fox H. Differential diagnosis of hydatidiform moles. Gen Diagn Pathol 1997; 143: 117-25.
14. Weinstein IB. Disorders in cell circuitry during multistage carcinogenesis: the role of homeostasis. Carcinogenesis 2000; 21: 857-64.
15. Kim KK, Shim JC, Kim JR. Overexpression of p21, cyclin E and decreased expression of p27 in DMBA (7, 12-dimethylbenzanthracene)-induced rat ovarian carcinogenesis. Pathol Int 2003; 53: 291-6.
16. van de Putte G, Holm R, Lie AK, Trope CG, Kristensen GB. Expression of p27, p21, and p16 protein in early squamous cervical cancer and its relation to prognosis. Gynecol Oncol 2003; 89: 140-7.
17. Al-Bozom IA. p53 and Bcl-2 oncoprotein expression in placentas

with hydropic changes and partial and complete moles. APMIS 2000; 108: 756-60.
18. Cheung AN, Srivastava G, Chung LP, *et al*. Expression of the p53 gene in trophoblastic cells in hydatidiform moles and normal human placentas. J Reprod Med 1994; 39: 223-7.
19. Cheville JC, Robinson RA, Benda JA. p53 expression in placentas with hydropic change and hydatidiform moles. Mod Pathol 1996; 9: 392-6.
20. Halperin R, Peller S, Sandbank J, Bukovsky I, Schneider D. Expression of the p53 gene and apoptosis in gestational trophoblastic disease. Placenta 2000; 21: 58-62.
21. Kale A, Soylemez F, Ensari A. Expressions of proliferation markers (Ki-67, proliferating cell nuclear antigen, and silver-staining nucleolar organizer regions) and of p53 tumor protein in gestational trophoblastic disease. Am J Obstet Gynecol 2001; 184: 567-74.
22. Lee YS. p53 expression in gestational trophoblastic disease. Int J Gynecol Pathol 1995; 14: 119-24.
23. Yang X, Zhang Z, Jia C, Li J, Yin L, Jiang S. The relationship between expression of c-ras, c-erbB-2, nm23, and p53 gene products and development of trophoblastic tumor and their predictive significance for the malignant transformation of complete hydatidiform mole. Gynecol Oncol 2002; 85: 438-44.
24. Marzusch K, Ruck P, Horny HP, Dietl J, Kaiserling E. Expression of the p53 tumour suppressor gene in human placenta: an immunohistochemical study. Placenta 1995; 16: 101-04.
25. Bamberger A, Sudahl S, Bamberger CM, Schulte HM, Loning T. Expression patterns of the cell-cycle inhibitor p27 and the cell-cycle promoter cyclin E in the human placenta throughout gestation: implications for the control of proliferation. Placenta 1999; 20: 401-6.
26. Cheung AN, Shen DH, Khoo US, Wong LC, Ngan HY. p21WAF1/CIP1 expression in gestational trophoblastic disease: correlation with clinicopathological parameters, and Ki67 and p53 gene expression. J Clin Pathol 1998; 51: 159-62.