p21 Gene Codon 31 Arginine/Serine Polymorphism: Non-Association With Endometriosis

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p21, an important regulator of the cell cycle, acts as a mediator of the growth-suppressing and -promoting functions of p53. We aimed to investigate the association between codon 31 polymorphisms of p21 gene and endometriosis. Women were divided into two groups: endometriosis (n = 102) and nonendometriosis (n = 119). The gene polymorphism for p21 codon 31 involved a base change from AGC to AGA and amino acid changes from serine (Ser) to arginine (Arg). Polymorphisms (Ser homozygotes, heterozygotes, Arg homozygotes) between both groups were detected and compared. Associations between the endometriosis

and polymorphisms were evaluated. The results revealed that the distributions of different p21 polymorphisms in both groups were nonsignificantly different. The proportions of Ser homozygote/heterozygote/Arg homozygote in endometriosis and nonendometriosis populations were 26.5/48.0/25.5% and 17.6/50.4/31.9%, respectively. We concluded the noncorrelation between the endometriosis and the p21 codon 31 polymorphism. p21 gene codon 31 arginine/serine polymorphism is not a useful marker for prediction of endometriosis susceptibility. *J. Clin. Lab. Anal.* 15:184–187, 2001. © 2001 Wiley-Liss, Inc.

Key words: codon 31 polymorphism of p21; endometriosis; polymorphism; p21; single nucleotide polymorphism

INTRODUCTION

Endometriosis, a multifactorial and complex disease, involves environmental and genetic factors (1). Endometriosis displays features similar to malignancy, including local invasion and aggressive spread to distant organs. Tumor suppressor genes, such as p53 and p21, play a role in the regulation of cell growth and prevention of carcinogenesis. There is a hypothetical connection between apoptosis and endometriosis (2). Altered tumor suppressor genes might be related with the development of endometriosis (3). Therefore, it is logical to suspect the correlation between p53/p21 polymorphism and the endometriosis formation.

p21 (also known as WAF1, Cip1, Sdi1, Mda 6, and Cap20) is a cyclin-dependent kinase inhibitor (CDK) upregulated by wild-type tumor suppressor protein p53 (4,5). p53 is the most common genetic change reported in human cancer. However, about half of cancers do not have p53 mutation (6). Therefore, mutations of genes situated upstream or downstream of p53 might have similar oncogenic effects (7). Numerous cancers are related to the abnormal p21 presentation, including the cervical carcinoma (8), ovarian carcinoma (9), bladder can-

cer (10), prostate cancer (11), hepatoma (12), colorectal carcinoma (7,13), lung cancer (14), brain tumor (15), oral carcinoma (16), nasopharyngeal carcinoma (17), esophageal carcinoma (18), breast cancer (19), lymphoma (20), etc. However, no literature revealed the association between the endometriosis and the p21 codon 31 polymorphism.

Only a few studies reported the relationships between p21 codon 31 polymorphisms and individual diseases. Li et al. (7) demonstrated the p21 gene codon 31 arginine/serine polymorphisms is not associated with a colorectal cancer predisposition. Koopmann et al. (15) demonstrated that the p53 mutation is not involved in the formation of brain tumors. In our laboratory, we have performed three surveys about the p21 codon 31 distribution in individual diseases, including bladder carcinoma, lung cancer, and urolithiasis. We observed

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that Arg form of codon 31 in p21 (Arg homozygotes or heterozygotes) is related with a poor prognosis in advanced lung adenocarcinoma (Hsia et al., unpublished data) and higher risk of bladder cancer (Chen et al., unpublished data). Arg homozygote is also related with less risk of urolithiasis formation (Chen et al., unpublished data).

Single nucleotide polymorphisms (SNPs) are the most abundant types of DNA sequence variation in the human genome (21). SNP is a single base pair on the DNA that varies from person to person. The SNP marker has gained more and more popularity for its quick, accurate, and inexpensive properties for the genetic analyses of different disease (22). The SNP marker provides a new method for identification of complex gene-associated diseases such as endometriosis. In this series, we first tried to detect the p21 gene codon 31 arginine/serine polymorphism in Taiwanese women with or without endometriosis. We aimed to evaluate whether p21 codon 31 polymorphism is a useful marker for prediction of endometriosis susceptibility. Ours is the first survey intended to make such an evaluation.

MATERIALS AND METHODS

Pre-menopausal Taiwanese women with surgically diagnosed endometriosis and those without were included. All patients were divided into two groups: endometriosis ($n = 118$) and nonendometriosis ($n = 140$). All women accepted the peripheral blood sampling for genotype analyses. The genomic DNA was prepared from peripheral blood by use of a DNA Extractor WB kit (Wako, Japan). This series was approved by the ethical committee of the China Medical College Hospital. Informed consent was obtained from all women who donated their blood.

The polymorphism for p21 codon 31 involved a C→A transversion at nucleotide 168 (AGC→AGA) change in the amino acid from serine (Ser) to arginine (Arg). Polymerase chain reactions (PCRs) were carried out to a total volume of 50 μ l, containing genomic DNA, 2–6 pmole of each primer, 1X Taq polymerase buffer (1.5 mM MgCl₂), and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The primer for p21 codon 31 was designed from codon 1 start (5'-GTCAGAACCGGCTGGGGATG-3') to codon 91 (5'-CTCCTCCCAACTCATCCC GG-3'), according to the procedure described by Li et al. (7). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer). The cycling condition for codon 31 was set as follows: one cycle at 94°C for 5 minutes, 35 cycles at 94°C for 30 seconds, 57°C for 40 seconds, and 72°C for 40 seconds, and one final cycle of extension at 72°C for 7 minutes.

The PCR product of 272 bp was mixed with 2 units Bsp I (New England Biolabs, Beverly, MA) and the reaction buffer according to the manufacturer's instructions. The reaction was incubated for 3 hours at 37°C. Then, 10 μ l of the product was

loaded into 3% agarose gel containing ethidium bromide for electrophoresis. The restriction site was designed to be located at the frequently seen allele of codon 31 (AGC) to form a cuttable site. Two fragments of 89 bp and 183 bp will be present if the product can be cut.

All polymorphisms were divided into Serine homozygote (Ser, cuttable), Arginine homozygote (Arg, uncuttable), and Ser/Arg heterozygote (half cut). The distributions of p21 polymorphisms in both groups were examined. Correlation between the p21 genotype and endometriosis was evaluated. The SAS system with χ^2 and logistic regression were utilized for statistical analyses. A P value < 0.05 was considered statistically significant.

RESULTS

The PCR products included a cuttable fragment (AGC, Ser homozygote, 89 bp and 183 bp), an uncuttable fragment (AGA, Arg homozygote, 272 bp), and a heterozygous form. The proportions of different p21 polymorphisms in both groups were nonsignificantly different (χ^2 value = 2.82) (Table 1). The proportions of Ser homozygote/heterozygote/Arg homozygote in endometriosis and nonendometriosis populations were 26.5/48.0/25.5% and 17.6/50.4/31.9%, respectively. There were nonsignificant differences between both groups in age, weight, and height. The frequency of Ser homozygotes in the endometriosis group (26.5%) was insignificant but higher than that in the control group (17.6%). The odds of individuals with heterozygotes and Pro homozygotes experiencing endometriosis are 0.64 and 0.53 times lower than the odds of individuals with Arg homozygotes (Table 1).

DISCUSSION

p21 gene, localized at the chromosome 6p21.2, is a gene that regulates and arrests the cell cycle (23). p21 is highly associated with cell death in the response to the wild-type tumor suppressor protein p53 (4,5). The activation of the p21 gene could inhibit the cyclin-dependent kinase complexes, which results in the inhibition of cell cycle from the G1 to the S phase (4,5). Altered p21 could interrupt the p53-mediated pathway of cell cycle and influence the progression of apoptosis.

There is controversy about the correlation between p21

TABLE 1. Distribution of p21 codon 31 polymorphisms in women with and without endometriosis

	Endometriosis (n = 102)	Nonendometriosis (n = 119)	χ^2 statistics	Odds ratio (95% confidence interval)
Ser/Ser	27 (26.5%)	21 (17.7%)	2.82 ^a	1.0
Ser/Arg	49 (48%)	60 (50.4%)		0.64 (0.32, 1.26)
Arg/Arg	26 (25.5%)	38 (31.9%)		0.53 (0.25, 1.14)

^aNonsignificant difference.

presentation and individual diseases. Pasz-Walczak et al. (13) demonstrated that p21 protein is involved in the regulatory mechanisms of carcinogenesis in colorectal cancer. Li et al. (8) demonstrated that the immunohistochemical expression of p21 is helpful for early detection and prognosis of cervical carcinoma. Cao et al. (24) demonstrated that the deregulation of p21 protein may affect the onset and metastasis of ovarian carcinoma. Chen et al. (19) demonstrated that the p21 is related with the activation of estrogen-signaling pathway in breast tumor. In contrast, Schneider et al. (14) demonstrated that serum p21 protein is not a useful marker for the early detection of lung cancer. Bankfalvi et al. (25) demonstrated the nonsignificantly prognostic value of immunohistochemical expression of p21 in breast carcinoma.

Numerous gene polymorphisms were related with endometriosis development, including the gene polymorphisms for estrogen receptors (ER), androgen receptors (AR), and p53. In our previous surveys, we noted that the ER, AR, and p53 codon 72 polymorphisms likely contribute to the pathogenesis of endometriosis (Hsieh et al., unpublished data). The 14 TA repeats for ER and 21 CAG repeats for AR are related with a higher risk of endometriosis. We noted that p53 Arg homozygotes are related to a lower risk of endometriosis development and the heterozygotes or Pro homozygotes are related to a higher risk of endometriosis formation.

In this series, we first survey the correlation between p21 codon 31 polymorphism and endometriosis, which revealed their nonassociation. This may be due to the fact that p21 expression could be activated through p53-dependent or p53-independent pathways (23). Although the real role of p21 upon endometriosis is unclear, it suggested that the p21 codon 31 could not become a useful genetic marker for endometriosis susceptibility. Furthermore, the roles of other p21 polymorphisms (such as at codon 14, 25, 32, 39, 64, or 149) (15,26) upon endometriosis development deserved further examination.

The distribution of p21 polymorphism in different diseases is unclear. Koopmann et al. (15) demonstrated that the most abundant p21 polymorphism is located at codon 31, which involved the base change from AGC to AGA and amino acid changes from Ser to Arg. In the surveys of the p21 polymorphisms in human brain tumors, they observed that the allelic frequencies for Ser- and Arg-form amino acids were 90.5 and 8.5 %, respectively (15). Li et al. (7) demonstrated the 18% of the Arg-form amino acid in colorectal cancer. Bahl et al. (26) and Li et al. (7) observed the 4 and 18% of the Arg-form amino acid in the patients with esophageal and colorectal cancer, respectively. In this series, we observed an Arg distribution in endometriosis patients of roughly 50%, which was different from that of Koopmann et al. (15). The difference of the Ser/Arg distribution may be due to racial or disease variations.

In conclusion, the correlation between endometriosis and p21 gene codon 31 does not exist. It appears that the p21

gene codon 31 Arg/Ser polymorphisms may not be useful genetic markers for the prediction of endometriosis susceptibility. This should be the basis of future surveys of the p21 polymorphism; the real role of the p21 polymorphism in endometriosis remains to be clarified. Furthermore, the role of other p21 polymorphisms in endometriosis development also merits further surveys.

REFERENCES

1. Bischoff FZ, Simpson JL. Heritability and molecular genetic studies of endometriosis. *Hum Reprod Update* 2000;6:37-44.
2. Meresman GF, Vighi S, Buquet RA, Contreras-Ortiz O, Tesone M, Rumi LS. Apoptosis and expression of Bcl-2 and Bax in eutopic endometrium from women with endometriosis. *Fertil Steril* 2000;74:760-766.
3. Jiang X, Hitchcock A, Bryan EJ, et al. Microsatellite analysis of endometriosis reveals loss of heterozygosity at candidate ovarian tumor suppressor gene loci. *Cancer Res* 1996;56:3534-3539.
4. el-Deiry WS, Tokino T, Waldman T, et al. Topological control of p21WAF1/CIP1 expression in normal and neoplastic tissues. *Cancer Res* 1995;55:2910-2919.
5. Gujuluva CN, Baek JH, Shin KH, Cherrick HM, Park NH. Effect of UV-irradiation on cell cycle, viability and the expression of p53, gadd153 and gadd45 genes in normal and HPV-immortalized human oral keratinocytes. *Oncogene* 1994;9:1819-1827.
6. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. *N Engl J Med* 1993;329:1318-1327.
7. Li YJ, Laurent-Puig P, Salmon RJ, Thomas G, Hamelin R. Polymorphisms and probable lack of mutation in the WAF1-CIP1 gene in colorectal cancer. *Oncogene* 1995;10:599-601.
8. Li X, Jiao S, Mi R. Clinical significance of p21H-ras expression and H-ras codon 12 mutation in squamous intraepithelial lesion (SIL) and carcinoma of uterine cervix. *Chung Hua Chung Liu Tsa Chih* 1997;19:306-308.
9. Levesque MA, Katsaros D, Massobrio M, et al. Evidence for a dose-response effect between p53 (but not p21WAF1/Cip1) protein concentrations, survival, and responsiveness in patients with epithelial ovarian cancer treated with platinum-based chemotherapy. *Clin Cancer Res* 2000;6:3260-3270.
10. Jahnson S, Karlsson MG. Tumor mapping of regional immunostaining for p21, p53, and mdm2 in locally advanced bladder carcinoma. *Cancer* 2000;89:619-629.
11. Cheng L, Lloyd RV, Weaver AL, et al. The cell cycle inhibitors p21WAF1 and p27KIP1 are associated with survival in patients treated by salvage prostatectomy after radiation therapy. *Clin Cancer Res* 2000;6:1896-1899.
12. Hsu SL, Chen MC, Chou YH, Hwang GY, Yin SC. Induction of p21(CIP1/Waf1) and activation of p34(cdc2) involved in retinoic acid-induced apoptosis in human hepatoma Hep3B cells. *Exp Cell Res* 1999;248:87-96.
13. Pasz-Walczak G, Kordek R. Comparative evaluation of the expression of cell cycle regulating proteins: cyclin D1, p53 and p21 (WAF1) in colorectal cancer. *Pol J Pathol* 2000;51:63-69.
14. Schneider J, Presek P, Braun A, Löffler L, Weitowitz HJ. Serum ras (p21) as a marker for occupationally derived lung cancer? *Clin Chem Lab Med* 2000;38:301-305.
15. Koopmann J, Maintz D, Schild S, et al. Multiple polymorphisms, but no mutations, in the WAF1/CIP1 gene in human brain tumors. *Br J Cancer* 1995;72:1230-1233.
16. Harada K, Ogden GR. An overview of the cell cycle arrest protein, p21(WAF1). *Oral Oncol* 2000;36:3-7.
17. Lin CS, Kuo HH, Chen JY, Yang CS, Wang WB. Epstein-barr virus nuclear antigen 2 retards cell growth, induces p21(WAF1) expression, and modulates p53 activity post-translationally. *J Mol Biol* 2000;303:7-23.

18. Woodward TA, Klingler PD, Genko PV, Wolfe JT. Barrett's esophagus, apoptosis and cell cycle regulation: correlation of p53 with Bax, Bcl-2 and p21 protein expression. *Anticancer Res* 2000;20:2427–2432.
19. Chen X, Danes C, Lowe M, Herliczek TW, Keyomarsi K. Activation of the estrogen-signaling pathway by p21(WAF1/CIP1) in estrogen receptor-negative breast cancer cells. *J Natl Cancer Inst* 2000;92:1403–1413.
20. Kanavaros P, Stefanaki K, Vlachonikolis J, et al. Expression of p53, p21/waf1, bcl-2, bax, Rb and Ki67 proteins in Hodgkin's lymphomas. *Histol Histopathol* 2000;15:445–453.
21. Kwok PY, Gu Z. Single nucleotide polymorphism libraries: why and how are we building them? *Mol Med Today* 1999;5:538–543.
22. Collins FS, Guyer MS, Chakravarti A. Variation on a theme: cataloging human DNA sequence variation. *Science* 1997;278:1580–1581.
23. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. *Nature* 1993;366:701–704.
24. Cao L, Yang Z, Li X. Expression and significance of tumor suppressor gene WAF1 in epithelial ovarian neoplasms. *Hunan I Ko Ta Hsueh Hsueh Pao* 1998;23:602–604.
25. Bankfalvi A, Tory K, Kemper M, et al. Clinical relevance of immunohistochemical expression of p53-targeted gene products mdm-2, p21 and bcl-2 in breast carcinoma. *Pathol Res Pract* 2000;196:489–501.
26. Bahl R, Arora S, Nath N, Mathur M, Shukla NK, Ralhan R. Novel polymorphism in p21 waf1/cip1 cyclin dependent kinase inhibitor gene: association with human esophageal cancer. *Oncogene* 2000;19:323–328.