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Research Paper

P53 codon 11, 72, and 248 gene polymorphisms in endometriosis

Yao-Yuan Hsieh [1 2], Chich-Sheng Lin [1]

1. Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan
2. Department of Obstetrics and Gynecology, China Medical University Hospital, Taichung, Taiwan

Correspondence to: Chich-Sheng Lin, Ph.D. Department of Biological Science and Technology, National Chiao Tung University, 75 Po-Ai Street, Hsinchu 300, Taiwan. Tel: 886-3-5131338, Fax: 886-3-5729288. E-mail: lincs@cc.nctu.edu.tw.

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Objective: Mutated p53 gene is related to the instability of cell growth and cell cycle progression. We aimed to evaluate the association between endometriosis and p53 codon 11, 72 and 248 gene polymorphisms.

Patients and methods: Women were divided into two groups: (1) moderate/severe endometriosis (n=148), and (2) non-endometriosis groups (n=150). P53 gene polymorphisms include codon11 Glu/Gln or Lys (GAG->CAG or AAG), codon 72 Arg/Pro (CGC->CCC), and codon 248 Arg/Thr (CGG->TCG). These gene polymorphisms were amplified by polymerase chain reaction and detected by electrophoresis after restriction enzyme (*Taq* I, *BstU* I, *Hap* II) digestions. Associations between the endometriosis and p53 polymorphisms were evaluated.

Results: The distributions of p53 codon 72 polymorphisms in both groups were significantly different. The proportions of Arg homozygotes/heterozygotes/Pro homozygotes in both groups were 9.5/66.2/24.3% and 30.7/50/19.3%. The proportions of Arg/Pro alleles were 42.6/57.4% and 56/44%. The distributions of p53 codon 11 and 248 polymorphisms in both groups were non-significantly different. All individuals appeared the wild genotypes (Glu11 and Arg248 homozygotes).

Conclusion: Association between endometriosis and p53 codon 72 polymorphism exists. P53 codon 72*Pro-related genotype and allele are related with higher susceptibility of endometriosis. P53 codon 11 and 248 polymorphisms are not related with endometriosis susceptibility.

Key words: Endometriosis, gene, polymorphism, p53, SNP

1. Introduction

Endometriosis, a common polygenic/multifactorial disease, might be caused by an interaction between multiple genes as well as the environment [1]. Endometriosis displays features similar to malignancy, including local invasion and aggressive spread to distant organs. Its monoclonal origin indicates the neoplastic and genetic natures of most endometriotic lesions [2]. Genomic alterations may represent important events in the development of endometriosis. Tumor suppressor genes play a role in the regulation of cell growth and prevention of carcinogenesis. The altered tumor suppressor genes might be related with the development of endometriosis [3].

p53, a representative tumor suppressor, is involved in cell proliferation and progression of various tumor types. There is discrepancy about this presentation of p53 polymorphisms and various tumors. The p53*Arg72 homozygote is considered to be a risk factor in the development of cancer [4]. In contrast, some investigators demonstrated the non-association between the different p53 polymorphisms and individual cancer development [5]; other studies revealed the higher risks in the individuals with p53*Pro72 homozygote [6,7]. Furthermore, some investigators also demonstrated the associations of individual disorders with p53 codon 248 (Table 5).

Scanty literature presented the association between the endometriosis and p53 polymorphism. High frequency of p53 locus deletion was observed in the endometriosis specimens [8]. The p53 protein abnormalities and chromosomal aberrations may be involved in malignant transformation of ovarian endometriosis [9]. In contrast, some investigators have demonstrated the undetectable expression of p53 in the endometriosis specimens [10-12]. To resolve these issues, we aimed to detect the p53 codon 11, 72, and 248 polymorphisms in Taiwanese women with or without endometriosis. To the best of our knowledge, this report is the largest survey in this aspect. Furthermore, it is also the first report about the distributions of p53 codon 11 and 248 polymorphisms in endometriosis.

2. Material and methods

Pre-menopausal Taiwanese women with surgically and histologically diagnosed endometriosis were included prospectively. All patients were divided into two groups: (1) moderate/severe endometriosis (n=119, according to revised American Fertility Society classification); and (2) non-endometriosis group (n=108). The non-endometriosis statuses were confirmed during the cesarean section or diagnostic laparoscopy. All operations were performed by same surgeon (Hsieh YY). All women accepted the peripheral blood sampling for genotype

analyses. There were non-significant differences between both groups in age, weight, and height. The experiment was approved by Ethical Committee and Institutional Review Board of the China Medical University Hospital. Informed consent was signed by all the women who donated their blood.

The genomic DNA was prepared from peripheral blood leukocytes by use of a genomic DNA isolation kit (Blossom, Taipei, Taiwan). A total of 50 ng genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 µl containing 10 mM Tris-HCL pH 8.3, 50 mM potassium chloride, 2.0 mM magnesium chloride, 0.2 mM each deoxyribonucleotide triphosphate, and 1 U DNA polymerase (Amplitag; Perkin-Elmer, Foster City, CA, USA).

The primer Pro72 was designed for p53 codon 72 in proline (Pro) form and Arg72 for arginine (Arg) form, according to the procedure described by Storey *et al*. [4]. P53codon 11 and 248 gene polymorphisms were determined according as the modified conditions of previous reports [13]. The SNP information for the genes involved was obtained through internet (http://www.ncbi.nlm.nih.gov/LocusLink/). The PCR conditions and restriction digestion of each SNP were listed in Table 1. The PCR amplification was performed in a programmable thermal cycler GenAmp PCR system 2400 (Perkin Elmer Applied Biosystems, Foster City, CA, USA). After PCR amplification, the individual gene polymorphisms were analyzed by gel electrophoresis of the PCR products after restriction enzyme digestions (New England Biolabs, Inc, Beverly, MA). The primer sequences, PCR conditions and related base pairs for the wild and mutant types were listed in Table 1.

Genotypes and allelic frequencies for p53 codon 11, 72 and 248 gene polymorphisms in both groups were compared. Correlations of these gene polymorphisms and endometriosis were evaluated. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS package (Version 8.1, SAS Institute Inc., Cary, North Carolina, USA) with χ^2 test was utilized for statistical analyses. A *p*-value of <.05 was considered statistically significant.

3. Results

Genotype proportions of different p53 codon 11 gene polymorphisms in both groups were non-significantly different (Table 2). All individuals appeared the wild genotype (Glu11) and allele. Proportions of p53*Glu homozygote/heterozygote (Glu/Gln, Glu/Lys)/Gln or Lys homozygote in both groups were: (1) 100/0/0% and (2) 100/0/0%, respectively. The proportions of Glu/Gln/Lys alleles in both groups were all 100/0/0%. There was no mutated genotype (p53 codon 11*Glu/Glu, Glu/Lys, Glu/Gln, Lys/Lys) observed in all individuals.

In contrast, the proportions of different p53 codon 72 genotypes in both groups were significantly different. The proportions of Arg homozygote/heterozygote/Pro homozygote in endometriosis and non-endometriosis populations were 9.5/66.2/24.3% and 30.7/50/19.3%, respectively (*p*-value=0.0001, Table 3). The distributions of Arg/Pro alleles in both groups were 42.6/57.4% and 56/44%, respectively (*p*-value=0.001, Table 3). There were increased numbers of Pro-related genotype (Pro homozygote and Arg/Pro heterozygote) and allele in endometriosis group compared to non-endometriosis group. It suggested the correlations between the Pro72 and endometriosis.

Genotype proportions of p53 codon 248 polymorphisms in both groups were also non-significantly different (Table 4). Proportions of p53 codon 248*Arg homozygote/heterozygote (Arg/Trp, Arg/Gln)/Trp or Gln homozygotes in both groups were: (1) 100/0/0% and (2) 100/0/0%, respectively. The proportions of Arg/Trp/Gln alleles in both groups were all 100/0/0% (Table 4). All individuals in both groups appear the wild (Arg248) genotype and allele. There was no mutated genotype (Trp, Gln) for p53 codon 248 observed in all individuals.

4. Discussion

Endometriosis is a common disorder in women, but its etiology remains unclear. The prevalence of endometriosis is 10% in the general population [14] and as high as 30-40% in infertile women [15]. Some heritable genetic defects might contribute to the development of endometriosis [16]. Somatic genetic alterations have been identified in endometriotic lesions, which might be related to its initiation and progression [17]. Kosugi *et al.* [18] demonstrated the increased heterogeneity and aneuploidy of chromosome 17 in endometriosis specimen. Because p53 is located in chromosome 17, the chromosome 17 aneuploidy might impair the function of p53, which influences the further progression of endometriosis.

The p53 gene and its encoded protein are related with the regulation of cell cycle, cellular growth, and apoptosis. It is a gatekeeper or guardian of the cell division [19]. The p53 mutations are associated with instability of cell development and cycle progression [20]. The wild-type p53 protein is a DNA-binding transcription factor that activates other tumor suppressor genes (e.g., p21, MDM2, GADD45, Bax), that are required for the regulation of cell cycle progression or apoptosis in response to DNA damage [21]. Alterations of p53 are related to the induction of apoptosis in malignant tumors.

Individuals lacking functional p53 might be associated with tumor development. Abnormal p53 presentation has been observed in some tumor specimens, including the cervical carcinoma [22], ovarian carcinoma [23], bladder cancer [24], prostate cancer [25], hepatoma [7], gastric cancer [26], lung cancer [7], brain tumor [27], esophageal carcinoma [28], breast cancer [29], lymphoma [30], etc. Mutated p53 gene or malfunctioned p53 protein has often observed in patients with most types of malignancies [20].

Single nucleotide polymorphisms (SNPs) provide a new way for the identification of complex gene-

associated diseases such as endometriosis. Tumor suppressor-related SNPs might directly or indirectly affect tumor progression as well as the further interruption of cell cycles. Allelic polymorphisms that occur in the regulatory regions of these tumor suppressor genes are closely associated with malignant changes. Reviewing MEDLINE database, we observed the correlation of numerous p53 gene polymorphisms with individual diseases (Table 5). However, few investigators demonstrated their correlation with endometriosis.

In fact, there are discrepancies about the distribution of p53 polymorphism in different malignancy. The p53 Arg72 homozygote is a significant risk factor in the development of invasive form of human papilloma virus-associated cancers [4]. In contrast, some investigators demonstrated the non-association between the cervical cancer and different p53 polymorphisms [5]. Furthermore, some reports even revealed that Pro72 homozygote is a risk factor of lung and hepatocellular carcinoma [7]. In the study of lung carcinoma, Wang *et al.* [35] found those patients with p53 Arg72 or Pro72 homozygous had worse prognoses compared with those with the heterozygous form.

Recently, Omori *et al.* [31] demonstrated that the non-association between the endometriosis and p53 codon 72 polymorphism. In their study, the proportions of Arg homozygotes/heterozygotes/Pro homozygotes in endometriosis and control groups were 35.2/48.6%/16.2% and 39.4/41.7/18.9 %, respectively. We can see their controls' distributions were compatible with ours. However, the percentage in endometriosis individuals is different from that of ours. This discrepancy may be due to the different endometriosis staging or racial variation. In this series, we observed that Arg72 homozygote is related with lower susceptibility of endometriosis development. The Pro forms of codon 72 in p53 (Pro homozygotes or heterozygotes) are related with the higher susceptibility of endometriosis development. Our finding was compatible with Wang *et al.* [7] and Yu *et al.* [6], who demonstrated the association between the Pro homozygotes and lung or hepatocellular carcinoma. Combined these above studies, it suggested the dominant p53* Pro forms is associated with the development of endometriosis in Taiwanese population.

Most cancer-related mutations of p53 are clustered in the four so-called 'hot spots', codon 175, 248, 273 and 281/282 [32]. Numerous reports presented the correlation statuses of p53 codon 248 polymorphism and individual diseases (Table 5). In this study, we noted the mutated somatic mutation of p53 condon 11 and 248 could not be observed in the peripheral lymphocytes from endometriosis populations. Therefore, these two SNPs will not become useful candidates for the suspecting the susceptibility of endometriosis. Presumably, the distinct biological condition caused by p53 genotype will be among various genetic, dietary, and environmental factors regulating hormonal and non-hormonal conditions in the development of endometriosis. These differences also reflect the etiological contributions of endogenous rather than exogenous factors to endometriosis. Furthermore, these polymorphisms might be in linkage disequilibrium with an unidentified functional polymorphism in p53 that influences endometriosis susceptibility.

In conclusion, the association between endometriosis and p53 polymorphism exists. The p53 Arg72 homozygotes are related with lower susceptibility of endometriosis development. The Pro72 homozygotes or heterozygotes are related with higher susceptibility of endometriosis development. The p53 codon 72 polymorphisms may become a useful marker to predict the endometriosis development. In contrast, the p53 codon 11 and 248 are not candidates for the useful marker of the endometriosis screening. Although the real role of p53 polymorphism has not been clarified, it deserves more attentions in the study of endometriosis and the development of gene therapy. However, the real roles of these p53 gene polymorphisms upon endometriosis remain to be clarified. Lager cohort recruitment is request for its further clarification. After the elucidation of these issues, some tumor suppressor gene polymorphisms might become useful markers to predict the future development of endometriosis as well as the development and intervention of genetic therapy.

Conflict of interests

The authors have declared that no conflict of interest exists.

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Figures and Tables

Table 1. The primer sequences and PCR conditions for p53 codon 11, 72 and 248 gene polymorphisms

Polymorphisms (locations)	Primers sequences (5′->3′)*	Denature (°C/sec)	Annealing (°C/sec)	Extention (°C/sec)	Restriction enzyme (°C/min)	SNP sequence	Allele (a.a.)	DNA fragment size (bp)
p53 codon 11	F-CTTGGGTTGTGGTGAAACATTG; R-GTCAGTCCCATGAATTTTCGCT	94/30	55/30	72/30	1 unit *Taq* I in 10 µL buffer at 65°C for 30 min	GAG (wild) CAG/AAG (mutant)	Glu Gln/Lys	239+140 379
p53 codon 72	F-TCCCCCTTGCCGTCCCAA; R-CGTGCAAGTCACAGACTT	95/30	58/30	72/45	1 unit *BstU*I in 10 µL buffer at 37°C for 30 min	CGC (wild) CCC (mutant)	Arg Pro	279 160+119
p53 codon 248 (exon 7)	F-TAGGTTGGCTCTGACTGTACCA; R-TGTGATGAGAGGTGGATGGGTA	94/30	58/30	72/30	1 unit *Hap* II in 10 µL buffer at 65°C for 30 min	CGG (wild) TGG/CAG (mutant)	Arg Trp/Gln	164+69 233

*F and R indicate forward and reverse primers

Table 2. Genotype and allele frequency of p53 codon 11 polymorphisms in populations with and without endometriosis.

	Endometriosis n=148 (%)	Non-endometriosis n=150 (%)	*p*-value
Genotype			NS
Glu/Glu	148 (100)	150 (100)	
Glu /Gln, Glu/Lys,	0	0	
Gln/Gln, Lys /Lys	0	0	
Allele frequency			
Glu	296 (100)	300 (100)	NS
Gln	0	0	
Lys	0	0	

Table 3. Genotype and allele frequency of p53 codon 72 polymorphisms in populations with and without endometriosis.

	Endometriosis n=148 (%)	Non-endometriosis n=150 (%)	*p*-value
Genotype			0.0001
Arg/Arg	14 (9.5)	47 (31.4)	
Arg /Pro	98 (66.2)	74 (49.3)	
Pro/Pro	36(24.3)	29 (19.3)	
Allele frequency			0.001
Arg	126 (42.6)	168 (56)	
Pro	170 (57.4)	132 (44)	

NS: non-significantly different

Table 4. Genotype and allele frequency of p53 codon 248 polymorphisms in populations with and without endometriosis.

	Endometriosis, n=148 (%)	Non-endometriosis, n=150 (%)	*p*-value
Genotype			NS
Arg/Arg	148 (100)	150 (100)	
Arg/Trp, Arg/Gln	0	0	
Trp/Trp, Gln/Gln	0	0	
Allele frequency			NS
Arg	296 (100)	300 (100)	
Trp	0	0	
Gln	0	0	

NS: non-significantly different

Table 5. Correlations of p53 codon 11, 72, and 248 gene polymorphisms with individual diseases

Correlation		Non-correlation	
SNP Location	Diseases and references	SNP Location	Diseases and references
Codon 11	Gastric cancer [33]	Codon 72	Gastric cancer [46] [a]
Codon 72*Arg	Gastric cancer [34] [a]	Codon 72	Cervical cancer [47,48] [b]
Codon 72*Arg	Cutaneous melanoma [35] [a]	Codon 72	Cervical cancer and human papillomavirus-related diseases [49] [b]
Codon 72*Pro	Lung cancer [36] [a]	Codon 72	Colorectal cancer [38] [a]
Codon 72*Arg	Lung cancer [37] [b]	Codon 72	Endometriosis [31] [a]
Codon 72*Arg	Breast cancer [38] [a]	Codon 72	Coronary artery disease [50] [a]
codon 248	Adrenal neoplasm [39] [b]	Codon 248	Pancreatic cancer [13] [b]
codon 248	Squamous cell carcinoma in eyes [40] [b]		
codon 248	Lung cancer [41] [b]		
codon 248	Ovarian cancer [42] [b]		
codon 248	Gastric carcinoma [43] [b]		
codon 248	Ulcerative colitis [44] [b]		
codon 248	Acute lymphoblastic leukemia [45] [a]		

[a]: Gene extracted from peripheral blood; [b]: Gene extracted from tumor tissue specimen.

Figure 1. Electrophoresis of p53 codon 11, 72, and 248. **(A)** p53 codon 11 (Marker 1. Glu homozygote; 2. Gln/Lys heterozygote) (*products of non-complete PCR reaction with primers). **(B)** p53 codon 72 (Marker 1. Arg/Pro heterozygosity; 2. Arg homozygosity; 3. Pro homozygosity). **(C)** p53 codon 248 (marker 1. Trp/Gln heterozygosity; 2. Arg homozygosity)