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Polymorphisms of p53 promoter and susceptibility to uterine leiomyoma

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Summary

Background: Uterine leiomyomas could be considered as benign tumor of human uterus smooth muscle with unknown etiology and pathophysiology.Furthermore, they are the most common indication of hysterectomy. The tumor suppressor gene p53 has been involved in various malignancies. Mutation in its promoter site may play a role in tumorigenesis of many malignancies including leiomyoma. *Materials and Methods:* For study of polymorphisms and allele frequency, 234 female patients with pathologically diagnosed uterine leiomyoma and 100 healthy blood donors as control group were assessed. DNAs were extracted from peripheral blood cells, amplified using polymerase chain reaction and restriction fragment length polymorphism (RFLP) technique was utilized for their analysis. *Result:* Proportions of A homozygote/heterozygote/G homozygote for SNP -250 A/G in leiomyoma group were 97.8%, 1.7%, and 0.4%, and in control group 97%, 3%, and 0%, respectively. In case of -216 T/C polymorphism, proportions of T homozygote/heterozygote/C homozygote in leiomyoma were 98%, 1.7%, and 0%, and in control samples 98%, 2%, and 0%, respectively. Genotype frequency of A homozygote/heterozygote/G homozygote for SNP-103 A/G was 97.9%, 1.7%, and 0.4% in leiomyoma group, and 98%, 2%, and 0% in control group, respectively. Proportions of A homozygote/heterozygote/G homozygote for SNP-33 A/G in leiomyoma group were 97.8%, 2.2%, and 0%, and 97%, 3%, and 0% in case samples, respectively. *Discussion:* Based on the present results in an Iranian female population, surprisingly there was no significant differences between leiomyoma cases and control samples regarding allele frequencies of p53 promoter polymorphism.Therefore, The p53 promoter polymorphism is not associated with the susceptibility of uterine leiomyomas in Iranian women.

Key words: Uterine leiomyoma; p53; Polymorphism; Iranian women.

Introduction

Uterine leiomyomas are benign clonal tumors of human uterus smooth-muscle cells. They are clinically apparent in about 25% of women [1] and with careful pathological examination of surgical specimens, the prevalence is as high as 77% [2]. Most of women with uterine leiomyomas are asymptomatic, thus often remain undiagnosed [3, 4]. Common signs and symptoms in symptomatic women may include pain, prolonged menstrual periods, bleeding between periods, pelvic or lower back pain, 'fullness' in the lower abdomen, with or without urinary or rectal symptoms, due to compression, and reproductive problems, such as infertility, multiple miscarriages, or early onset of labor during pregnancy [5]. The pathophysiology of uterine leiomyomas is not well understood. However, genetic predisposition, as well as steroid hormone concentrations, have a role in formation and growth of these tumors, as do growth factors important in fibrotic processes and angiogenesis [6].

One of the newest and useful means to study the etiology of polygenetic disorders with complex inheritance patterns is the determination of single nucleotide polymorphisms (SNPs) that is used in polygenetic disorders such as diabetes, hypertension, and neoplasms. The phenotypic effects of SNPs are based on direct genetic effects, gene-gene interactions, and gene-environment interactions [7]. Identification and cataloging of SNPs represents a major task for molecular biology in the near future [8]. Polymorphisms are defined as mutations with an allele frequency of at least 1% in a given population [9, 10]. Humans are believed to carry over a million distinct SNPs [10], with around 30,000 of them exerting clinically visible phenotypic effects [7]. Various mechanisms such as enhanced/reduced transcription, altered post-transcriptional or post-translational activities, or changes in the tertiary structure of the gene product may cause these effects [7, 10]. In this regard, the identification of genetic factors that predispose individuals to uterine leiomyoma could provide further insight into the etiology of this benign neoplasm.

As a tumor suppressor protein, p53 function is based on its ability to up- or downregulate the expression of many

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genes involved in cell growth, cell cycle progression, DNA repair, cellular senescence, autophagy, metabolism, and p53 regulation [11, 12]. Genomic instability of p53 plays a role in the development and progression of various tumor types, including cervical breast cancer [13], carcinoma [14], esophageal carcinoma [15], lung cancer [16], ovarian carcinoma [17], prostate cancer [18], brain tumor [19], bladder cancer [20], nasopharyngeal carcinoma [21], hepatoma [22], oral carcinoma [23], gastric cancer [24], and lymphoma [25]. Also, some researchers have investigated the undetectable expression of p53 in the leiomyoma specimen [26-30].

Functional inactivation of tumor suppressor genes during tumor progression has been shown to occur by either coding region mutation or promoter region. The segregation, substitution, or deletion within the promoter consequences might directly or indirectly interfere with the guarantee of p53 as well as the consequent tumorigenesis. Transcriptional inactivation of the promoter region may participate in carcinogenesis [31]. Transcriptional repression by p53 promoter methylation might contribute to tumor progression [32]. Therefore, mutations in p53 promoter region might play a partial role in the process of tumorigenesis. Furthermore, the understanding of the detailed characterization of p53 promoter is useful for the elucidation of the underlying regulation of p53 expression.

In a study Hsieh *et al.* found four SNPs in promoter of p53 protein (-250 A/G, -216 T/C, -103 A/G, and -33 A/G) with higher frequency among other variants in promoter of p53 protein in women with leiomyoma [33].

In this study the authors attempted to elucidate the relationship between high frequency polymorphisms of p53 promoter gene and leiomyoma. In reviewing the literature, few investigators demonstrated the mutation statuses of p53 promoter as well as its promoter region in leiomyoma individuals [33, 34]. Only one literature revealed the association between the leiomyoma and the p53 promoter genes [33] and there is no current study conducted in Iranian women.

Materials and Methods

In this study the authors analyzed four highly suspected SNPs in p53 promoter that might be cause of aberrant methylation promoter regions of p53 by the method of restriction fragment length polymorphism (RFLP) in leiomyoma in Iranian women. These SNP included -250 A/G, -216 T/C, -103 A/G, and -33 A/G. In this case-control study, premenopausal Iranian women with pathologically diagnosed leiomyomas and non-leiomyomas were included. Women without leiomyoma were examined by ultrasonography. All women were divided into two groups: (group 1) leiomyoma (n = 234) and (group 2) non-leiomyoma (n = 100). All women did not use hormone therapy in the past one year. Also pregnant women, smokers, postmenopausal women and women with estrogen-related cancers were excluded. After obtaining approval from the ethics committee of Shahrekord University of Medical Sciences, blood samples from all of the individuals were collected. Genomic DNA was extracted from peripheral blood samples with phenol–chloroform extraction method. About 25 ng of genomic DNA was mixed with ten pmole of PCR primers in a total volume of 25 ml containing 10 mMTris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM in each deoxyribonucleotide triphosphate, and one unit of Tag DNA polymerase.

PCR primers were synthesized according to the published p53 GenBank promoter sequence (accession no. X54156). A The sequences of the primers were as following: Forward, 5'-GAT CCA GCT GAG AGC AAA CG-3'; Reverse, 5'-CTT ACC CAA TCC AGG GAA GC-3'. The PCR amplification was performed in a PCR machine with the following PCR conditions: one cycle at 95°C for six minutes, 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 40 seconds, and final cycle of extension at 72°C for six minutes.

In analyzing by the method of RFLP, the PCR product (464 bp) digestion was performed according to commercial instructions of restriction enzymes. The related enzymes and DNA fragments length are listed in Table 1. Genotypes for p53 promoter polymorphisms in the leiomyoma and control groups were compared. For statistical analyses, SPSS statistics, version 16.0-with Chi-square tests were utilized. A p-value < 0.05 was considered statistically significant.

Table 1. — *Restriction enzymes and DNA fragments after digestion for p53 promoter -250 A/G, -216 T/C, -103 A/G, and -33 A/G polymorphisms.*

SNP	Restriction enzyme	Allele type	DNA fragments (bp)
-250 A/G	Bgl I	WT	464
		MT	363+101
-216 T/C	Mae I	WT	355+109
		MT	137+218+109
-103 A/G	Tau I	WT	464
		MT	250+214
-33 A/G	Dde I	WT	464
		MT	316+148

Table 2. — *Genotype distribution of -250 A/G polymorphism of p53 promoter.*

	Leiomyoma n=232 (%)	Non-leiomyoma n=100 (%)	p-value
Genotype			0.614
AA	227 (97.8)	97 (97)	
AG	4 (1.7)	3 (3)	
GG	1 (0.4)	0 (0)	
Allele			
A	458 (98.7)	197 (98.5)	
G	6 (1.3)	3 (1.5)	

Results

In this study 234 women with uterine leiomyoma and 100 controls were examined. Mean age in groups were 44.5 ± 5.7 (leiomyoma) and 33.2 ± 9.3 (non-leiomyoma) ($p < 0.01$). Genotype distribution of different p53 polymorphisms in both groups was not significantly different. Proportions of A homozygote/heterozygote/G homozygote for SNP -250 A/G in leiomyoma group were 97.8%, 1.7%, 0.4%, and in non-

Table 3. — *Genotype distribution of SNP -216 T/C polymorphism of p53 promoter.*

	Leiomyoma n=234 (%)	Non-leiomyoma n=100 (%)	*p*-value
Genotype			0.885
TT	230 (98)	98 (98)	
TC	2 (1.7)	4 (2)	
CC	0 (0)	0 (0)	
Allele			
T	462 (99.6)	198 (99)	
C	2 (0.4)	2 (1)	

Table 4. — *Genotype distribution of SNP -103 A/G polymorphism of p53 promoter.*

	Leiomyoma n=234 (%)	Non-leiomyoma n=100 (%)	*p*-value
Genotype			0.794
AA	229 (97.9)	98 (98)	
AG	4 (1.7)	2 (2)	
GG	1 (0.4)	0 (0)	
Allele			
A	462 (98.7)	198 (99)	
G	6 (1.3)	2 (1)	

Table 5. — *Genotype distribution of SNP -33 A/G polymorphism of p53 promoter.*

	Leiomyoma n=230 (%)	Non-leiomyoma n=100 (%)	*p*-value
Genotype			0.654
AA	225 (97.8)	97 (97)	
AG	5 (2.2)	3 (3)	
GG	0 (0)	0 (0)	
Allele			
A	455 (98.9)	197 (98.5)	
G	5 (1.1)	3 (1.5)	

leiomyoma group 97%, 3%, and 0%, respectively (Table 2). In -216 T/C polymorphism, proportions of T homozygote/heterozygote/C homozygote in leiomyoma group were 98%, 1.7%, and 0%, and in non-leiomyoma group 98%, 2%, and 0%, respectively (Table 3). Genotype frequency of A homozygote/heterozygote/G homozygote for SNP -103 A/G in leiomyoma group 97.9%, 1.7%, and 0.4% and in non-leiomyoma group 98%, 2%, and 0%, respectively, (Table 4) and proportions of A homozygote/heterozygote/G homozygote for SNP -33 A/G in leiomyoma Group were 97.8%, 2.2%, and 0%, and in non-leiomyoma group 97%, 3%, and 0%, respectively (Table 5).

Discussion

Human uterine leiomyomas (fibroids) are common benign neoplasms in reproductive age and premenopausal women. These tumors have a significant and increasingly health concern for a large amount women throughout the world. Alternative therapies are few, and the treatment of choice by many physicians is hysterectomy. There is interesting evidence suggesting that the development of leiomyomas may be influenced by many factors [35], including acquired ones such as hypertension, obesity, and early menarche and they may also be associated to genetic changes [36]. Understanding of the genetic tendency for this benign tumor and the specific genes that are dysregulated may indicate new possibilities for pharmaceutical intervention and ultimately lead to strategies for gene therapy and prevention. Primary studies have begun on identification of genes through a genome-wide scan for finding possible mechanisms of gene therapy that take advantage of the physiology of leiomyomas [6, 37].

Aberrant DNA methylation and its related transcriptional aberration were associated with cancer processes, which may show an important primary mechanism that triggers transformation of a single tumor stem cell that will finally develop into a leiomyoma tumor [36]. Understanding the role of apoptosis in the normal regression of myometrial tissue and how its failure may influence on tumorigenesis may help to develop effective and less invasive treatment modalities for this disease [38]. P53 gene and its encoded protein are related with the regulation of cellular growth, cell cycle, and apoptosis. It is a gatekeeper or guardian of cell division [39, 40]. The p53 mutations are associated with instability of cell development and cycle progression [41]. Somatic mutations in p53 gene are the most common genetic alterations found in human malignancies [42] and are detected in approximately half of all cancers. Thus it is reasonable to suppose that genetic changes in the p53 promoter region might determine an individual susceptibility to leiomyoma.

Mutation in promoter regions of the gene is associated with transcriptional inactivation of various tumor suppressor genes in neoplasms [31]. Thus, mutations in p53 promoter region might play a partial role in the process of tumor genesis. Few reports are available on mutations in the p53 promoter in cancers. In contrast, Kullmann *et al.* demonstrated that analyzing the sequence p53 promoter did not reveal any mutational base change in the rheumatoid arthritis synovial fibroblasts [43]. This indicates that in these patients, p53 mutations in synovial fibroblasts do not contribute to the proliferative and aggressive behavior of these cells. Also Nayak and Das reported the absence of mutations and deletions in p53 promoter in breast tumorigenesis [34]. In fact, the specific mutation pattern of p53 gene appears different expression depending on the types of tissue [43].

The present authors could not find any significant difference between frequency of -250 A/G and -33 A/G, (similar to Hsieh *et al.*[33]), but also frequency of -216 T/C and -103 A/G appeared with similar distributions between Iranian women with and without leiomyoma. It seems to be in contrast with an analysis in premenopausal Tai-

wanese women with surgically diagnosed leiomyoma [33], which suggested that alleles of -216 T/C, -103 A/G within the promoter region of p53 genes were associated with higher susceptibility of leiomyoma development. On the other hand, some studies suggested that the sex steroids influence the growth of leiomyomas by stimulating cell proliferation rather than by affecting apoptosis and no difference in apoptotic index was observed between leiomyomas and normal myometrium [26]. Growth modulation of leiomyomas by hormone deprivation might occur via mechanisms independent of apoptosis [44]. This theory also could confirm the present results.

Conclusion

The present study indicates that p53 promoter polymorphisms including -250 A/G, -216 T/C, -103 A/G, and -33 A/G are not associated with an increasing risk of uterine leiomyoma in Iranian women. The authors suggested sequencing the p53 promoter region in Iranian women with leiomyoma. In order to detect the novel sequence variations and determine whether mutations in transcription regulatory sequences of p53 gene may result in leiomyoma development or pathogenesis of leiomyoma could be considered a p53 independent and novel study.

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