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MDM2 SNP309 (rs2279744) and p53 Codon Arg72Pro (rs1042522) SNP in Cervical Carcinogenesis

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Abstract

To investigate the association between polymorphisms (SNP) in the p53 and murine double minute 2 homolog (MDM2) promoter 309 in cervical carcinogenesis. SNP at p53 codon 72 polymorphisms and MDM2 promoter 309 (T/G) together with human papillomavirus (HPV) types were examined in a total of 187 cervical smear samples using real time PCR. 27 cases with HPV types 16 and/or 18 had significantly higher frequency of the TG + GG genotype and G allele than 56 with other types of high-risk HPV (P = 0.0136). 48 cases with HPV types 52 and/or 58 had significantly higher frequency of the TG + GG genotype and G allele than 56 with other types of high-risk HPV (P = 0.001). Our studies have demonstrated that the frequency of G allele in MDM2 promoter 309 increased from LSIL to HSIL and that there was an increased OR for G allele in HSIL cases with high-risk HPV types including 52 and 58. It is known that geographically different oncogenic HPV types 52 and 58 are more prevalent than 16 and 18 in a Japanese population.

Keywords

p53, MDM2, Polymorphism, HPV, Cervical Carcinogenesis

1. Introduction

Cervical cancer is the second most common cancer in women worldwide, and is a both preventable and curable disease especially if identified at an early stage. It is widely accepted that specific human papillomavirus (HPV) types are the central etiologic agent of cervical carcinogenesis. Other environmental and host factors also play

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decisive roles in the persistence of HPV infection and further malignant conversion of cervical epithelium [1]. Although many previous reports have focused on HPV and environmental factors [2] [3], the role of host susceptibility to cervical carcinogenesis is largely unknown.

The intracellular level of p53 is regulated through an auto-regulatory feedback loop in which p53 induces transcription of murine double-minute 2 homologue (MDM2), a gene that encodes an ubiquitin protein ligase that regulates the stability of p53 by targeting it for proteasomal degradation. The most important role of MDM2 protein is the regulation of p53 [4]-[6]. The functions of MDM2 and p53 are linked through an auto-regulatory negative-feedback loop that maintains low p53 protein levels in the absence of stress [7]. However, this feedback loop is disrupted in many human tumors that contain somatic p53 mutations. A polymorphism at codon 72 of the p53 gene results in the substitution of arginine (Arg) for proline (Pro) in the gene product. It has been suggested that the homozygous Arg genotype increased the susceptibility of p53 protein to degradation by E6 protein derived from oncogenic HPV [8].

A recent study has shown that a single nucleotide polymorphism (SNP) in the MDM2 gene promoter, SNP309 (a T to G change at nucleotide 309 in the first intron), increases the affinity of the promoter for the transcription activator Sp1, resulting in higher level of MDM2 mRNA and MDM2 protein and a subsequent attenuation of the p53 pathway [9]. SNP309 occurs at a relatively high frequency in the general population and has been shown to be associated with accelerated tumorigenesis and the timing of cancer onset [10]-[12].

Although many studies have identified environmental factors associated with cervical cancinogenesis, the interaction between environmental and genetic factors remains to be elucidated. Previous reports have shown a direct relationship between increasing proliferation and progressive derailment of p53 and MDM2 [13] [14], however, there have been very few reports on the correlation between SNP of MDM2 gene and cervical cancer susceptibility [15]. In the present study, we investigated a polymorphism at codon 72 of the p53 gene and MDM2-SNP309 together with HPV types in exfoliated cervical cell samples from the patients with squamous intraepithelial lesion (SIL) of the cervix, and evaluated the biological significance of this genotype in cervical carcinogenesis in a Japanese population.

2. Materials and Methods

2.1. Cell Sample

We conducted genotype analysis of MDM2-SNP309 together with HPV typing in a total of 187 cervical smear samples obtained from patients who received cervical cancer screening. They consist of 52 normal, 94 low-grade SIL (LSIL), and 41 high-grade SIL (HSIL). All of 187 subjects were Japanese women who visited Osaka Center for Cancer and Cardiovascular Diseases Prevention in the past 10 years. Cervical cell samples from these patients were collected from the uterine ectocervix and the endocervical canal by cotton swabs, placed in phosphate-buffered saline, and stored at −200˚C until use. Final histologic diagnosis was confirmed by colposcopy-directed biopsy for the patients with abnormal cytology. The protocol of this study was approved by our institutional review board and all samples were obtained with informed consent.

2.2. DNA Preparation

The exfoliated cervical cells or cell lines were disrupted with lysis buffer [20 mM NaCl, 10 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% SDS, 50 μg/ml proteinase K], and genomic DNA was extracted with phenol-chloroform and precipitated with ethanol using standard techniques.

2.3. Genotyping of p53 Codon 72 and MDM2-SNP309

The single-nucleotide polymorphisms (SNPs) were genotyped using pre-designed TaqMan® allelic discrimination assays (RefSNP accession MDM2 SNP309 (rs2279744) and the TP53 Arg72Pro (rs1042522) SNPs) in a 7900HT Real-Time polymerase chain reaction (PCR) System from Applied Biosystems under conditions recommended by the manufacturer (Foster City, CA, USA).

2.4. HPV Typing

The presence of various HPV types was examined using L1-PCR according to the method reported by Nagano

et al. [16]. Briefly, 100 ng of cellular DNA was subjected to PCR in the presence of published consensus primers (L1C1 and L1C2) [17]. Amplified HPV fragments were typed on the basis of the RFLP among HPVs. Initial typing of amplified HPV fragments was performed by digestions with RsaI, DdeI, and then confirmed by digestions with several additional restriction enzymes as described previously [16] [17]. L1-PCR can detect 22 registered low-risk (6, 11, 34, 40, 42, 43, 44) and high-risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 69) HPV types.

2.5. Statistical Analysis

To compare the polymorphic features of genotype and HPV status between normal, LSIL and HSIL groups, Fisher's exact test or Pearson's chi-square test was used. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

Table 1 shows the frequency of Amino acid at p53 codon 72 in exfoliated cervical cell samples in 187 samples examined. No statistical difference was found in the genotype frequency of Amino acid at p53 codon 72 between SILs and controls among 187 patients with or without high-risk HPV. **Table 2** shows the frequency of MDM2-SNP309 in exfoliated cervical cell samples in 187 samples examined. No statistical difference was found in the genotype frequency of MDM2-SNP309 between SILs and controls among 187 patients with or without high-risk HPV.

Table 1. HPV status and frequency of Amino acid at p53 codon 72 in exfoliated cervical cell samples.

Study group	n	Amino acid at p53 codon 72		OR	95% CI	P value
		Arg/Arg	Arg/Pro + Pro/Pro			
High-risk HPV −						
Normal	42	20 (47.6%)	22 (52.4%)	1		
LSIL	72	26 (36.1%)	46 (63.9%)	1.608	0.74 - 3.48	0.227
HSIL	11	4 (36.7%)	7 (63.3%)	0.989	0.26 - 3.69	0.987
High-risk HPV +						
Normal	10	3 (30.0%)	7 (70.0%)	1		
LSIL	22	10 (45.5%)	12 (54.5%)	0.514	0.10 - 2.52	0.409
HSIL	30	14 (46.6%)	16 (53.4%)	0.952	0.31 - 2.87	0.931

Table 2. HPV status and frequency of MDM2-SNP309 in exfoliated cervical cell samples.

Study group	n	Genotype at MDM2-SNP309		OR	95% CI	P value
		TT	TT + GG			
High-risk HPV −						
Normal	42	8 (19.0%)	34 (81.0%)	1		
LSIL	72	13 (18.0%)	59 (82.0%)	0.41	0.09 - 17.55	0.895
HSIL	11	4 (36.7%)	7 (63.3%)	0.38	0.09 - 1.51	0.186
High-risk HPV +						
Normal	10	3 (30.0%)	7 (70.0%)	1		
LSIL	22	8 (36.3%)	14 (63.7%)	0.75	0.15 - 3.74	0.725
HSIL	30	3 (10.0%)	27 (90.0%)	5.14	1.17 - 22.49	0.021

Table 3 shows the high-risk 16, 18, 52, 58 types and frequency of Amino acid at p53 codon 72 in exfoliated cervical cell samples in 187 samples examined. No statistical difference was found in the genotype frequency of Amino acid at p53 codon 72 between HPV types 16, 18, 52, 58 and other High-risk types among 62 patients with high-risk HPV.

Interestingly, 27 cases with HPV types 16 and/or 18 had significantly higher frequency of the TG + GG genotype and G allele than 56 with other types of high-risk HPV (P = 0.0136) as indicated in **Table 4**. Also interestingly, 48 cases with HPV types 52 and/or 58 had significantly higher frequency of the TG + GG genotype and G allele than 56 with other types of high-risk HPV (P = 0.001) as indicated in **Table 4**.

4. Discussion

MDM2 is the key negative regulator of p53 and dysfunction of these genes that may be associated with an increased rate of accumulation of genetic errors thereby enhancing the progression of the disease. A functional T to G polymorphism in the promoter region of the MDM2 gene (MDM2-SNP309) has been reported to profoundly accelerate tumor formation suggesting that it may also represent a powerful cancer predisposing allele [9]-[12].

Meissner *et al*. [15] reported that no statistically significant association was observed between SNP309 and cervical cancer. In contrast, Arvanitis and Spandidos [18] demonstrated that MDM2 gene expression was found to be up-regulated in SIL by RT-PCR assay. Our previous results using exfoliated cervical cell samples demonstrated that there was an increased OR for TG + GG genotype in HSIL cases compared to controls among the patients with high-risk HPV. Our present study also observed that HPV types 16, 18, 52 and 58, the most prevalent and aggressive types worldwide, are predominant in cases with TG + GG genotype and G allele. These observations suggest that MDM2-SNP309 and high-risk HPV infection may be cooperatively associated with cervical carcinogenesis.

Recently, Cristina *et al*. [19] investigated the role of HPV infection in oral carcinogenesis and demonstrated that overexpression of MDM2 protein is closely associated with high-risk HPV-related oral lesions. Cescon, D.W. *et al*. [20] reported that p53 Arg72 Pro Pro/Pro was associated with a 2-fold increased risk of death in all esophageal cancers, whereas MDM2 T309G G/G was associated with a 7-fold increased risk of death in esophageal squamous cell carcinoma. Singhal, P. *et al*. [21] reported that on gene-gene interactions between MDM2 and p53 polymorphisms, the frequency of MDM2 G/G and p53 Arg/Arg together was found to be 6.5-fold higher in cervical cancer patients compared with healthy controls.

There is a possibility that MDM2-SNP309 and MDM2 overexpression may play an important role not only in early stage of cervical carcinogenesis but also in progression and metastasis of invasive cervical squamous cell

Table 3. High-risk 16, 18, 52, 58 HPV types and frequency of p53 codon 72 in exfoliated cervical cell samples.

Study group	Amino acid at p53 codon 72	
	Arg/Arg	Arg/Pro + Pro/Pro
HPV types 16, 18 (n = 27)	11 (40.7%)	16 (59.3%)[a]
HPV types 52, 58 (n = 48)	23 (47.9%)	25 (52.1%)[b]
HPV HR other types (n = 56)	28 (50.0%)	28(50.0%)[a,b]

P = 0.4870 χ^2 vs HPV types 16, 18 VS HPV other types; P = 0.8470 χ^2 vs HPV types 52, 58 VS HPV other types.

Table 4. High-risk 16, 18, 52, 58 HPV types and frequency of MDM2-SNP309 in exfoliated cervical cell samples.

Study group	Genotype at MDM2-SNP309	
	TT	TT + GG
HPV types 16, 18 (n = 27)	2 (7.5%)	25 (92.5%)[a]
HPV types 52, 58 (n = 48)	3 (6.3%)	45 (93.7%)[b]
HPV HR other types (n = 56)	18 (32.2%)	38(67.8%)[a,b]

P = 0.0136 χ^2 vs. HPV types 16, 18 VS HPV other types; P = 0.001 χ^2 vs. HPV types 52, 58 VS HPV other types.

carcinomas. It would be of interest to further examine molecular correlation between MDM2-SNP309, MDM2 gene expression and p53 status in cervical cells infected by high-risk HPV.

Previous studies [22] have demonstrated that high-risk HPV infection is inversely correlated with apoptosis of cervical epithelial cells and that a decrease of apoptosis is closely associated with higher histologic grade of SIL. Moreover, MDM2 gene expression was reported to be significantly up-regulated in SIL. Further studies on the differential gene expression profiles between normal cervical keratinocytes and cervical cancer cell lines with or without G allele at MDM2 gene promoter 309 may provide the better understanding for the effect of this SNP in the sequence of cervical carcinogenesis. Moreover, it might be of interest to further examine whether cultured cervical cancer cells with TG or GG genotype could escape from apoptosis in response to MDM2 overexpression and subsequent p53 dysfunction.

In the present study, we demonstrated the role of MDM2-SNP309 in cytologic materials from women with premalignant cervical disease. MDM2 gene promoter polymorphism may be closely associated with cervical carcinogenesis in a Japanese population particularly in high-risk HPV group. Our studies have demonstrated that the frequency of G allele in MDM2 promoter 309 increased from LSIL to HSIL and that there was an increased OR for G allele in HSIL cases with high-risk HPV types including 52 and 58. It is known that geographically different oncogenic HPV types 52 and 58 are more prevalent than 16 and 18. Takehara [23] reported that HPV 52 and 58 detection rates were higher than that observed for HPV 16 in a Japanese population. These observations are potentially important in managing SIL patients by cytologic examination and in understanding the pathogenesis of cervical cancer. It would be of interest to further evaluate whether this polymorphism could be used as a disease marker for the natural history of cervical neoplasias in a setting of longitudinal cohort study and for the determination of appropriate screening interval in patients with or without high-risk HPV.

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