行政院國家科學委員會專題研究計畫 成果報告

基質細胞衍生因子(SDF-1)及其受體(CXCR4)在子宮頸上皮內贅瘤及子宮頸癌的表現
研究成果報告(精簡版)

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Polymorphisms of human Stromal cell-derived factor-1 gene and neoplastic lesions of uterine cervix

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Abstract

Backgrounds: To investigate the association of stromal cell-derived factor-1 (SDF-1) gene polymorphisms with the neoplastic lesions of uterine cervix in Mid-Taiwan women. *Methods*: 498 blood samples were collected from 161 patients with neoplasia of uterine cervix, including 76 cancer patients and 61 patients with high-grade dysplasia, 24 low-grade dysplasia, and 337 normal women. Stromal cell-derived factor-1 (SDF-1) gene was selected. *Results*: Compared with homozygotes GG, we found the heterozygote genotype, GA has no significantly different distribution between patients with cervical neoplasia and normal women (*P* = 0.539). Moreover, compared with cancer patients and patients with high-grade dysplasia and low--grade dysplasia, women with GG also have no significantly different distribution. *Conclusions*: SDF-1 A gene polymorphism could be not considered as a factor related to an increased susceptibility of neoplasia of uterine cervix.

Keywords: Stromal cell-derived factor-1 gene; single nucleotide polymorphisms; neoplasia of uterine cervix

1. Introduction

Cervical intraepithelial neoplasia (CIN) is regarded as a precancerous lesion that may progress to invasive carcinoma. In the Bethesda System, low-grade squamous intraepithelial lesions (LSILs) include CIN 1, which is pathologically compatible with mild dysplasia (Low-grade dysplasia). High-grade squamous intraepithelial lesions (HSILs) include CIN 2 and CIN 3, which are pathologically compatible with moderate dysplasia as well as severe dysplasia and carcinoma in situ, separately and are regarded as high-grade dysplasia. CIN 1 lesions have a high rate of clinical regression and around 60% of low-grade dysplasias resolve without the need for treatment [1-3]. CIN 1 by itself is not regarded as an obligate precursor to cervical cancer [4]. Meta-analysis studies of cervical dysplasia natural history estimated that 22% of CIN 2 lesions would progress to CIN 3 without treatment [1]. Women with CIN 2 are at substantial risk for cervical cancer. However, CIN 2 lesions can arise without a detectable CIN 1 phase. It indicates that low-grade and high-grade lesions can be independent processes [4, 5]. CIN 3 more often persists or progresses to cancer and regression will be less common. Therefore, these high-grade dysplasia lesions are accepted as the pathological states those precede invasive cervical cancer. High-grade dysplasia and invasive cancer can be regarded as true neoplastic lesions of uterine cervix, while low-grade lesions are not. In

previous studies, nm23-H1 is reported to be correlated with tumor progression of uterine cervix [6-9]. High expression of nm23-H1 was demonstrated in high-grade dysplasia and invasive cancer of uterine cervix.

Over expressions of stromal cell-Derived Factor-1 (SDF-1; CXCL12), the C-X-C (Cys-Xxx-Cys) subfamily of chemokine [10] and its seven-transmembrane G-coupled receptor, CXCR4, are found in human hepatoma cells [11-17] and are significantly associated with the progression of various cancer [14, 15, 17]. As well, SDF-1 can reorganize human hepatoma cell cytoskeleton [13] and activate matrix metalloproteinase-2, -9 , which benefit the invasion, adhesion, and migration [11, 13] of cancer.

SDF-1 gene is located on chromosome 10q 11.1 [18] and a single nucleotide polymorphism (SNP), a guanine to adenine (G→A), at position 801 of the 3'-untranslated gene region results in a SDF-1 chemokine gene polymorphism (rs1801157) is found [18, 19]. The SDF-1 A/A or AG gene polymorphism has been suggested to alter the production of SDF-1 [20, 21 and is involved in the development of carcinogenesis [22-36]. We hypothesized that the variant gene polymorphisms of SDF-1 could contribute to the susceptibility and clinicopathological development of cervical intraepithelial neoplasia and cervical cancer, and those could be considered as useful information to improve the selection of susceptible individuals at higher risk of

developing cervical intraepithelial neoplasia and cervical cancer. However, the role of SDF-1 gene polymorphisms in cervical intraepithelial neoplasia and cervical cancer has not been clarified. The purpose of this study is to investigate the relationship between single nucleotide polymorphisms of SDF-1 a genes and cervical intraepithelial neoplasia and cervical cancer risk, and whether this polymorphism could influence the clinicopathological status of cervical intraepithelial neoplasia and cervical cancer

2. Materials and methods

2.1. Population

76 patients with cancer of uterine cervix and 61 with high-grade dysplasia and 24 with low-grade dysplasia were enrolled into this study at the Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taiwan. In the meanwhile, 337 healthy women, who did not have cervical lesions, were included as age group matched controls to achieve a control-to-case ratio of 2 to 1. The mean ages and standard deviation of women with cervical neoplasia (cervical cancer or high-grade dysplasia) and normal women were47.9 $\pm$ 12.9 and 44.5 $\pm$ 10.3 years old, respectively. All of them lived in Mid-Taiwan. The patients with cervical

high-grade dysplasia or invasive cancer had received treatment at the Department of Obstetrics and Gynecology in Chung Shan Medical University Hospital between January 2007 and July 2009. Cervical cancer patients were staged IB or IIA clinically based on the classification of the International Federation of Gynecology and Obstetrics. Cancer patients underwent abdominal radical hysterectomy and pelvic lymph nodes dissection between January 2007 and July 2009. HSIL patients received abdominal total hysterectomy, vaginal total hysterectomy, or large loop excision of transformation zone. Their specimens were histopathologically compatible with high-grade dysplasia, ie, moderate or severe dysplasia, or carcinoma in situ. If the cases had discrepancy between cytologic and histologic diagnosis, they were excluded in this study. One hundred and twenty-two blood samples were collected form patients with neoplastic lesions of uterine cervix. Two hundred and forty-four blood samples were collected from women, who were found to have normal cervical Papanicolaou smear. They received the smear at Out Patient Department in Chung Shan Medical University Hospital and the normal cytologic diagnosis was further supported using colposcopy. Neither SIL lesions nor cancers cells were found from their cervical smears. The study was performed with the approval by Institutional Review Board, Chung Shan Medical University Hospital, and informed consent was obtained from each subject.

2.2. Blood samples collection and genomic DNA extraction

Venous blood samples were drawn from each subject and placed into Vacutainer tubes containing EDTA and stored at 4℃. Genomic DNA was extracted from buffy coats (white blood cells) using a QIAamp DNA blood mini kits (Qiagen, Valencia, USA) according to the manufacture's protocol. DNA was dissolved in TE buffer [10mM Tris (PH 7.8), 1mM EDTA] and then quantitated by a measurement of OD260. Final preparation was stored at -20℃ and used as templates in polymerase chain reaction (PCR)

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

The SDF-1 polymorphism was determined by PCR-RFLP assay. The sequences of primers used to amplify the SDF-1 genotype were 5′-CAGTCAACCTGGGCAAAGCC-3′ and 5′-CCTGAGAGTCCTTTTGCGGG-3′ [23]. PCR was performed in a 10 μl volume containing 100 ng DNA template, 1.0 μl of 10X PCR buffer (Invitrogen, Carslbad, CA, USA), 0.25 U of Taq DNA polymerase (Invitrogen), 0.2 mM dNTPs (Promega, Madison, WI, USA) and 200 nM of each primer (MDBio Inc. Taipei, Taiwan). The PCR cycling conditions were 5 min at

94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 2 min at 72 °C, with a final step at 72 °C for 20 min to allow a complete extension of all PCR fragments (20). PCR products of SDF-1 gene polymorphism were subjected to enzymatic digestion by incubation with *HpaII*, for 4 hr at 37^{O}C and then submitted to electrophoresis in 3 % agarose gels. For SDF-1, the wild type homozygous alleles (G/G) yielded a 100 and 193-bp products, the heterozygous alleles (G/A) yielded 100-, 193- and 293-bp products, while the mutated type homozygous alleles (A/A) yielded a 293-bp product. The results were shown in Figure 1.

2.6. Statistical analysis

The distribution ofSDF-1 SNPs in patients with cervical neoplasia and healthy women was analyzed using Chi-square test. A significance difference was defined by $P < 0.05$.

3. Results

The frequencies of SDF-1 gene polymorphisms were studied in161 patients with neoplasia of uterine cervix patients and compared to 337 healthy controls. In our recruited healthy control group, the allele frequencies were 30.6 % and 69.4 % for A allele and G allele, respectively, of SDF-1 gene polymorphism. These frequency

distributions were similar to healthy Japanese population. The PCR-RFLP products are shown in Figure 1.

The frequency of SDF-1 genotypes showed no significant difference (p=0.539) between 161 patients with neoplasia of uterine cervix patients and healthy controls (Table 1). We furthermore classified the individuals with at least one mutated allele as one subgroup and regarded the individuals with homozygous wild type alleles as another subgroup of SDF-1. For the SDF-1 gene polymorphism, no significant difference was observed in any allele frequency distribution (Table 2).

As well, we estimated SDF-1 gene polymorphisms on the 76 cervical cancer patients, 61 patients with high-grade dysplasia and 24 low--grade dysplasia. We found that no significant difference was found between SDF-1 genotypes and 76 cervical cancer patients, 61 patients with high-grade dysplasia and 24 low--grade dysplasia (Table 3). Moreover, no significant association between control and 76 cervical cancer patients, 61 patients with high-grade dysplasia and 24 low--grade dysplasia in any allele frequency distribution were observed (Table 4).

4. Discussion

Over expressions of SDF-1 or interaction between the two chemokines are associated with the development and metastasis of cancer [11-17]. In addition, single

nucleotide polymorphism of SDF-1-3'A is considered associated with the alteration of SDF-1 production [20, 21] and cancer risk [22-26]. However, in this study, we found no significant difference of genotypic frequencies of SDF-1 gene polymorphism between healthy controls and cervical cancer patients.

To our knowledge, Hirata et al. [21] demonstrated that individuals with AA or AG genotype of SDF-1 were significantly associated with higher expression of SDF-1 and CXCR4. SDF-1 produced in lymph nodes contributed to lymph node metastasis in CXCR4 expressing carcinoma cells via ERK1/2 or PI3K-Akt/PKB pathway [27-29], and suggested that over expression of CXCR4 in carcinoma cells benefited to acquiring lymph node metastatic potential [27], as well, the exhibition of SDF-1/CXCR4 autocrine loop was associated with the distant metastasis and poor prognosis of cancer. However, individuals with 3'A of SDF-1 gene polymorphism could increase the expression of both SDF-1 and CXCR4 [21], consequently, increased the risk of cancer and induced poorly pathological development of HCC. However, further investigations are required to evidence the comparison of both two chemokine expressions with their polymorphisms in cervical cancer.

The limitation in our study is that we did not obtain the information of risk factors which are considered as the risk factors of cervical cancer in our recruited healthy control group because it was difficult to request healthy individuals response

to those information or collected those data during their health examination. This limitation maybe limit the adjustment of those confoundings.

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Figure Legends

Figure 1. Polymerase chain reaction-restriction fragment length polymorphism of SDF-1 gene. PCR products of SDF-1 gene polymorphism were subjected to enzymatic digestion by incubation with *HpaII* for 4 hr at 37^{O}C and then submitted to electrophoresis in 3 % agarose gels. Wild type homozygous alleles (G/G) yielded a 100 and 193-bp products, the heterozygous alleles (G/A) yielded 100-, 193- and 293-bp products, while the mutated type homozygous alleles (A/A) yielded a 293-bp product.

Table 1 Genotypes distribution of SDF-1 single nucleotide polymorphisms in patients with cervical neoplasia and normal women

SDF-1 polymorphisms	Normal women	Patients with cervixal neoplasia	*P* value
	N=337 (%)	N=161 (%)	
GG	164 (48.7%)	74 (46 %)	
GA	140 (41.5 %)	66 (41%)	0.539
AA	33 (9.8 %)	21 (13 %)	
GG/GA	164 (48.7%)	74 (46 %)	0.320
AA	173 (51.3%)	87 (54 %)	

Table 2 Alleles distribution of SDF-1 single nucleotide polymorphisms in patients with cervical neoplasia and normal women

SDF-1 polymorphisms	Normal women	Patients with cervixal neoplasia	*P* value
	N=674 (%)	N=322 (%)	
G	468 (69.4%)	214 (66.5 %)	
A	206 (30.6 %)	108 (33.5 %)	0.344

Table 3. Genotypes distribution of SDF-1 single nucleotide polymorphisms in patients with LSILs, HSILs, cervical cancer and normal women

SDF-1 polymorphisms	GG	GA	AA	*P* value	GG/GA	*P* value
normal women	164	140	33		304	
N=337 (%)	(48.7%)	(41.5 %)	(9.8 %)		(90.2%)	
LSILs	11	8	5	0.223	19	0.157
N=24 (%)	(45.9%)	(33.3 %)	(20.8 %)		(79.2%)	
HSILs	26	29	6	0.659	65	1.000
N=61 (%)	(42.6%)	(47.6 %)	(9.8 %)		(90.2%)	
Cervical cancer	37	29	10	0.654	66	0.406
N=76 (%)	(48.7%)	(38.2 %)	(13.1 %)		(86.9%)	

LSILs: Low-grade squamous intraepithelial lesions

HSILs: High-grade squamous intraepithelial lesions

Table 4. Alleles distribution of SDF-1 single nucleotide polymorphisms in patients with LSILs, HSILs, cervical cancer and normal women

SDF-1 polymorphisms	G	A	*P*
Normal women N=674 (%)	468 (69.4%)	206 (30.6 %)	
LSILs N=48 (%)	30 (62.5%)	18(37.5 %)	0.334
HSILs N=122 (%)	81 (66.4%)	41(33.6 %)	0.524
cervical cancer N=152 (%)	103 (67.8%)	49(32.2 %)	0.698

Figure 1