Gynecologic Oncology 119 (2010) 70–75

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Significant association of genetic polymorphism of human nonmetastatic clone 23 type 1 gene with an increased risk of endometrial cancer

Po-Hui Wang [a,b,c,*], Yu-Chiao Yi [d,e], Hsiu-Ting Tsai [f,g], Yi-Torng Tee [a,b,c], Jiunn-Liang Ko [h], Chih-Ping Han [c,i], Yu-Fan Liu [j], Long-Yau Lin [a,b,c,*], Shun-Fa Yang [c,k,l,*]

[a] Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan
[b] School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[c] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[d] Department of Obstetrics and Gynecology, Taichung Veterans General Hospital, Taichung, Taiwan
[e] Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan
[f] School of Nursing, Chung Shan Medical University, Taichung, Taiwan
[g] Department of Nursing, Chung Shan Medical University Hospital, Taichung, Taiwan
[h] Institute of Medical and Molecular Toxicology, Chung Shan Medical University, Taichung, Taiwan
[i] Clinical Trial Center, Chung Shan Medical University Hospital, Taichung, Taiwan
[j] Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan
[k] Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
[l] Department of Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 2 April 2010

Keywords:
Human nonmetastatic clone 23 type 1 gene
Single nucleotide polymorphisms
Allele
Endometrial cancer
Heterozygote

ABSTRACT

Objective. To investigate the association of single nucleotide polymorphisms (SNPs) of nonmetastatic clone 23 type 1 (nm23-H1) gene with endometrial cancer and their implication in clinicopathologic characteristics of women in Taiwan.

Methods. Three hundred and fifty-nine blood samples were collected from 268 healthy women and 91 patients with endometrial cancer to analyze SNPs rs16949649 and rs2302254 of nm23-H1 promoter using real time polymerase chain reaction and genotyping. The association of genotype and allele differences of nm23-H1 SNPs with endometrial cancer and their implication in some clinicopathologic variables were analyzed using Pearson's Chi-square or Fisher exact tests.

Results. Women with heterozygous genotypes TC in rs16949649 or CT in rs2302254 exhibited higher risk to develop endometrial cancer as compared to those with their wild-type or homozygous genotypes (odds ratio 3.30 and 1.86; 1.84 and 1.90 for respective SNP). Individuals with CC genotype were at less risk (OR: 0.08; $P=0.037$) to have non-endometrioid type as compared to those with TT genotype in rs16949649. However, a trend of increased risk (OR: 26.67; $P=0.01$) of advanced stage endometrial cancer (stage III–IV) was observed in patients with TT genotype as compared to those with CC genotype in rs2302254.

Conclusions. Heterozygous genotypes TC in rs16949649 and CT in rs2302254 of nm23-H1 promoter are potential susceptibility factors for endometrial cancer in Taiwan women. Once having the endometrial cancer, Taiwan women with variant homozygote CC in rs1694964 were at less risk to have non-endometrioid type, while women with variant homozygote TT in rs2302254 tended to have advanced stage cancer.

Introduction

Cancer of uterine corpus is the eighth common type of malignancy in women and the second common cancer of the lower genital tract in Taiwan. In 2007, Bureau of Health Promotion of the Department of Health reported the age standardized incidence rate for uterine cancer to be 8.3 per 100000 women in Taiwan. Endometrial cancer accounts for most of cancer of uterine corpus. The incidence of endometrial cancer is escalating in this country recently. It can be further categorized into endometrioid (type 1) and non-endometrioid (type 2) adenocarcinoma.

In 1988, Steeg et al. utilized differential colony hybridization to analyze seven cell lines derived from a murine K-1735 melanoma with varying metastatic potentials and found that the clone 23, exhibiting highest RNA levels, was associated with less metastatic potentials [1]. Then, they isolated a novel gene and termed it as nonmetastatic clone 23 gene, which was abbreviated to nm23 gene. Thereafter, Rosengard et al. identified the first human equivalent by screening a human fibroblast

* Corresponding authors. Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan. Fax: +886 4 24723229.
E-mail addresses: ming84921345@yahoo.com.tw (P.-H. Wang), ginhow84921344@yahoo.com.tw (L.-Y. Lin), ysf@csmu.edu.tw (S.-F. Yang).

0090-8258/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygyno.2010.06.013

cDNA library and referred it as nm23-H1 (human nonmetastatic clone 23 type 1) in 1989 [2]. At least nine human nm23 gene isotypes are discovered to date, among which nm23-H1 is regarded as the most representative one [3–5]. Nm23-H1 was demonstrated to be a metastasis-associated gene in various tumors [6–9]. The expression of nm23-H1 was found inversely proportional to the metastasis of breast cancer and colon adenocarcinoma [6,7]. In contrast, elevated nm23-H1 is associated with a significant reduction in survival for osteosarcoma and neuroblastoma patients [8,9]. Our previous study found that nm23-H1 expression was significantly associated with depth of stromal invasion in cancer of uterine cervix [10]. Its expression was highly related to overall survival of cervical cancer patients. In endometrial cancer, a trend of low protein level of nm23-H1 was found in those patients with lymph node involvement [11].

Two single nucleotide polymorphisms (SNPs) in the 5′ promoter region of the nm23-H1 gene have been documented in Chinese HapMap database. To our knowledge, no study reports the potential association of the nm23-H1 polymorphisms in 5′ promoter region with endometrial cancer. We purposed that SNPs in nm23-H1 promoter may influence its binding with transcription factors and affect promoter activity as well as gene transcription. Different SNPs therefore have different impacts on the expression of gene and then protein and exhibit different risk to develop endometrial cancer. In this study, we investigated the influence of the nm23-H1 gene polymorphisms on the susceptibility of endometrial cancer and their relationship with cancer stage and pathologic type of women in Taiwan.

Materials and methods

Population

Ninety-one patients, who were confirmed having endometrial cancer by pathologic report, were enrolled from the Department of Obstetrics and Gynecology, Taichung Veterans General Hospital and Chung Shan Medical University Hospital, Taiwan between April 1, 2005 and October 31, 2007. Meanwhile, 268 women without endometrial lesions were included as age group matched controls to achieve a control-to-case ratio of 3 to 1. The age (mean ± SD) of women with endometrial cancer and control women were 58.2 ± 10.5 and 56.2 ± 10.1 years old, respectively. The patients with endometrial cancer had received treatment at the Department of Obstetrics and Gynecology in Taichung Veterans General Hospital and Chung Shan Medical University Hospital between April 1, 2005 and October 31, 2007. They were staged according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) Classification and categorized into endometrioid (type 1) and non-endometrioid (type 2) adenocarcinoma histologically. Ninety-one blood specimens were collected from these patients. Two hundred and sixty-eight blood specimens were collected from women who visited the same hospitals for health examination with no history or suggestive clinical evidence of endometrial pathology. The study was performed with the approval by Institutional Review Boards of Taichung Veterans General Hospital and Chung Shan Medical University Hospital. Informed consents were obtained from all subjects.

Selection of nm23-H1 polymorphisms

dbSNP database has documented 12 SNPs in the 5′ promoter region of the nm23-H1 gene and provided the information of minor allele frequencies (MAFs) for five of them [12]. In order to have adequate power to evaluate the potential association, we investigated rs16949649 (−1465 T/C), rs3760468 (−1242 A/T), rs3760469 (−1181 T/G), and rs2302254 (−873 C/T), those with MAFs ≥ 5% [12]. Because rs16949649 is in strong linkage disequilibrium with both rs3760468 ($R^2 = 1$ and $D' = 1$) and rs3760469 ($R^2 = 0.967$ and $D' = 0.985$), based on Chinese HapMap data, rs16949649 may be analyzed as the proxy of the remaining two SNPs [12]. Therefore, we selected two SNPs, rs16949649 (−1465 T/C) and rs2302254 (−873 C/T) to analyze based on the study of Qu et al. [12] and Chinese HapMap.

Blood samples collection and genomic DNA extraction

Genomic DNA was extracted from EDTA anti-coagulated venous blood using a QIAamp DNA blood mini kits (Qiagen, Valencia, USA) based on the manufacture's protocol. DNA was dissolved in TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA] and then measured with a reference to OD260. Final preparation was stored at −20° C and used as templates in real time polymerase chain reaction (PCR).

Single nucleotide polymorphisms using real time polymerase chain reaction and genotyping

The extracted DNA was used as templates for real time PCR. The allelic discrimination of the nm23-H1 polymorphisms was assessed with the ABI Perkin Elmer Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA), using the TaqMan assay (assay IDs: C_34107066_10 for rs16949649 and C_2646888_1_ for rs2302254). The final volume for each reaction was 5 μL, containing 2.5 μL TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 0.125 μL primers/TaqMan probes mix, and 10 ng genomic DNA. The real time PCR reaction contained an initial denaturation step at 95° C for 10 min, followed by 40 cycles, each consisting of 92° C for 15 s and 60° C for 1 min. The fluorescence level was measured with the ABI PRISM 7900HT sequence detector (Applied Biosystems). Allele frequencies were determined by ABI SDS software.

Statistical analysis

The genotype and allele difference in distribution of nm23-H1 SNPs in patients with endometrial cancer and healthy women was analyzed using Pearson's Chi-square test by WinPepi Software, version 10.0. Odds ratio (OR) and its 95% confidence interval (CI) were estimated via the calculation of Chi-square test by the same software. We analyzed the difference between wild-type homozygotes and non-wild-type genotypes; between homozygotes and heterozygote; as well as between variant homozygote and non-variant genotypes for SNPs in patients with endometrial cancer and control women. Hommel's method was utilized to adjust the P values in response to multiple analyses by WinPepi software, version 10.0. A significant difference was defined by $P < 0.05$. We evaluated the risk of genotypic distribution of nm23-H1 SNPs associated with clinical characteristics (age, pathologic types and stages) using Fisher exact test by WinPepi software. Power is calculated using Primer software.

Results

Association of nm23-H1 polymorphisms with endometrial cancer

Ninety-one endometrial cancer patients were enrolled from a Taiwanese population. Seventy-seven patients were diagnosed to have endometrioid type of endometrial cancer, confirmed by pathologic report, whereas 14 patients had non-endometrioid type. Sixty-six patients were classified to stage I; 13 patients, stage II; 10 patients, stage III and 2 patients, stage IV (Table 1). The distribution of nm23-H1 SNPs in patients with endometrial cancer and normal women is shown in Table 2. We found that there are significant differences for SNPs distribution in rs16949649 ($P < 0.001$) and rs2302254 ($P = 0.026$) between women with endometrial cancer and normal women (Table 2). When wild-type homozygous genotype TT in rs16949649 was used as a reference, the variant homozygote CC and heterozygote TC exerted more risk to develop endometrial cancer (OR: 3.39 and 3.30, respectively; Table 2). However in rs2302354, only heterozygous