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Genetic polymorphism of the tissue inhibitor of metalloproteinase-1 is associated with an increased risk of endometrial cancer

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ABSTRACT

Background: Tissue inhibitors of metalloproteinases (TIMPs) are a family of multifunctional proteins known to possess a broad range of biological activities involved in tumorgenesis and mRNA expression of TIMP family members has been shown to be upregulated in numerous cancers and correlates with clinical outcomes. We investigated the association of TIMP-1 and TIMP-2 gene polymorphism with risk of endometrial cancer.
Methods: In the present case–control study, we enrolled a total of 118 endometrial cancer patients confirmed by histopathology and 229 unrelated healthy individuals. Polymorphism for TIMP-1 (_372C>T) and TIMP-2 (_418G>C and _303C>T) genes was genotyped by PCR-RFLP.
Results: Frequency of TIMP-1_372C/C genotype and 372-C allele differed significantly between patients with endometrial cancer (38.1% and 56.4% respectively) and healthy individuals (22.7% and 44.1% respectively). Individuals with TIMP-1_372 C/C genotype were at higher risk of endometrial cancer (OR = 2.37; 95%CI: 1.33–4.22). Stratification analysis showed that individuals with TIMP-1_372 C/C genotype were at increased risk for endometrioid (OR = 2.46; 95% CI 1.34–4.53) and low stage (stages I–II) endometrial cancer (OR = 3.24; 95% CI 1.22–4.13). However, no significant differences in TIMP-2_418G>C and TIMP-2_303C>T genotypes were observed between endometrial carcinoma cases and controls.
Conclusion: Individuals with TIMP-1_372C/C genotype were at significantly higher risk of endometrial cancer.

1. Introduction

Endometrial carcinoma is a common gynecological malignancy of the female urogenital tract. Biological evidence supports a role for estrogen as an endometrial carcinogen [1,2], yet little is known about the molecular events underlying tumor development and/or progression. Transformation and spread of malignant tumors is a multi-step process and many of these steps require degradation or breakdown of the extracellular matrix and connective tissue surrounding tumor cells. The major proteins involve in degradation of extracellular matrix are matrix metalloproteinases (MMPs) and Tissue inhibitors of metalloproteinases (TIMPs). The matrix metalloproteinases (MMPs) are a family of structurally related enzymes that can degrade the components of the extracellular matrix (ECM). It is believed that overexpression of these proteolytic enzymes in the tumors renders the cells capable of breaking down the ECM and basement membrane (BM), which under normal conditions maintain the integrity of the tissues, thereby allowing cells to invade the surroundings. MMPs are counteracted by the tissue inhibitors of metalloproteinases (TIMPs), which inhibit MMP activity and thereby restrict ECM breakdown. The balance between MMPs and TIMPs plays an important role in maintaining the integrity of healthy tissues and disturbed balance is associated with the malignant progression of cancer cells.

Previous studies have reported association of the MMPs and TIMPs polymorphisms in a number of different cancers. With respect to

Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases.

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endometrial cancer, there were two published reports on the association between the MMP-1 and MMP-9 polymorphisms and endometrial cancer risk in Japanese women [3,4], but no study about TIMPs polymorphism and endometrial cancer risk was reported. Given the important roles of TIMPs on tumor growth and progression, we conducted the present hospital-based, case–control study to assess the role of TIMP-1_372C>T, TIMP-2_418G>C and TIMP-2_303C>T polymorphism on endometrial cancer risk.

2. Materials and methods

2.1. Subjects

A total of 118 patients (age: 54.7 ± 10.6 y) confirmed by pathology to have endometrial cancer, and 229 healthy control individuals were enrolled in this study. The endometrial cancer patients were consecutively recruited from women with histologically confirmed endometrial cancer who presented for treatment at the Taichung Veterans General Hospital between April 1, 2005 and March 31, 2006, yielding a 93% participation rate. They were classified according to the International Federation of Gynecology and Obstetrics (FIGO) stage and histologically into endometrioid carcinoma and non-endometrioid carcinoma. Patients were excluded from the study if the operative records were unavailable or if there was any doubt about the diagnosis. Control samples consisted of 229 women (52.9 ± 8.7 y) recruited from individuals who visited the same hospital for health examination with no history, or suggestive clinical evidence, of endometrial pathology. Informed consent was obtained from all the women who enrolled in this study. The research protocol was approved by the human research board of the ethical committee of Taichung Veterans General Hospital.

2.2. Genotyping

Genomic DNA was extracted from EDTA anti-coagulated venous blood using the Wizard DNA purification kit (Promega, Madison, WI). The genotypes were analyzed by PCR-based techniques described in Table 1 [5].

2.3. Statistical analysis

Statistical analysis was performed using the SPSS12.0 software package (SPSS Company, Chicago, IL, USA). The χ^2 test was used for any deviation from Hardy–Weinberg equilibrium. The odds ratios (OR) and 95% confidence intervals (CI) for TIMP-1 and TIMP-2 genotypes and alleles in endometrial cancer case and control groups were calculated by logistic regression, a probability level of 5% was considered significant.

3. Results

Table 2 displays the clinical characteristics of 118 endometrial carcinoma cases from a Taiwanese population. The genotyping of the TIMP-1_372T>C, TIMP-2_418G>C and TIMP-2_303C>T polymorphisms was successful in all cases and controls studied. The distributions of alleles in all groups fit the Hardy–Weinberg equilibrium as shown in Table 3.

The genotype distributions of the polymorphisms of TIMP-1_372T>C, TIMP-2_418G>C and TIMP-2_303C>T in endometrial carcinoma cases and controls are shown in Table 4. The genotype of TIMP-1_372 T/T, T/C and C/C was 25.4%, 36.4% and 38.1% respectively in endometrial cancer cases and 35.8%, 41.5% and 22.7% respectively in controls. The presence of the C allele and C/C genotype frequency in the TIMP-1_372 genotype among endometrial cancer cases was higher than in normal controls. The TIMP-1 C allele was associated with a 1.68-fold (95% CI 1.22–2.31) and the TIMP-1_372 C/C genotype was associated with 2.37-fold (95% CI 1.33, 4.22) endometrial cancer risk than the TIMP-1 T allele and TIMP-1_372 T/T genotype respectively. Besides, no significant differences in TIMP-2_418G>C and TIMP-2_303C>T genotypes were observed between endometrial carcinoma cases and controls. To further evaluate the effect of TIMP-1_372T>C, TIMP-2_418G>C and TIMP-2_303C>T on endometrial carcinomas, the endometrial carcinoma cases were stratified with clinic–pathological characteristics and associations with TIMP-1_372T>C, TIMP-2_418G>C and TIMP-2_303C>T genotypes were analyzed (Table 5). Individuals with TIMP-1_372 C/C genotype were at an increased risk for patients over 50 years old (OR = 2.66; 95% CI 1.37–5.17), endometrioid type (OR = 2.46; 95% CI 1.34–4.53) and low stage (stages I–II) endometrial cancer (OR = 3.24; 95% CI 1.22–4.13). There was also a trend of increased risk observed in patient less than 50 years old, non-endometrioid and high stage endometrial cancer (OR = 1.86, 1.89 and 3.15 respectively), but no statistical significance was detected.

We also wondered whether the two different polymorphisms in TIMP-2 (TIMP-2_418G>C and TIMP-2_303C>T) can exert additive effect, and evaluated the frequency of combination of these two polymorphisms in endometrial cancer patients and healthy controls (Table 6), still no statistical significance was noted in different combinations between endometrial cancer patients and healthy controls.

4. Discussion

Tissue inhibitors of metalloproteinases (TIMPs) family, currently comprising four members (TIMP-1, -2, 3- and -4), are a family of multifunctional proteins numbered in order of their discovery and characterized by a conserved structure ranging from M_r 20,000 to 30,000 that inhibits the proteolytic activity of matrix metalloproteinases (MMPs) by forming non-covalent complexes with MMPs [6,7]. The balance between MMPs and TIMPs plays an important role in maintaining the integrity of healthy tissues and disruption of this balance may result in diseases associated with excessive ECM degradation, such as arthritis, cardiovascular diseases, nephritis, neurological disorders and cancer.

Several groups demonstrated that either over-expression of TIMPs or i.p. injection of recombinant TIMP-1 reduced experimental metastasis formation [8–10] and downregulation of both TIMP-1 and TIMP-2 expression is associated with increased invasiveness in tumors of various origins [11,12]. On the contrary, the cell growth-promoting effects of TIMP-1 and TIMP-2 have been observed in a wide

Table 1
Primer sequences and PCR conditions for amplification of TIMP SNPs.

SNP	Method	Primer	Sequences	Location	Annealing	BP	Enzyme
TIMP-1_372T>C	PCR-RFLP	Forward	GCACATCACTACCTGCAGTC	Exon 5	54 °C, 35 cycles	175	BssSI
		Reverse	GAAACAAGCCCACGATTTAG	phe 124 phe			
TIMP-2_418G>C	PCR-RFLP	Forward	CGTCTCTTGTTGGCTGGTCA	Promotor	64 °C, 35 cycles	304	BsoBI
		Reverse	CCTTCAGCTCGACTCTGGAG				
TIMP-2_303C>T	PCR-RFLP	Forward	TAGGAACAGCCCCACTTCTG	Exon 3	60 °C, 35 cycles	119	TspRI
		Reverse	CCTCCTCGGCAGTGTGTG	ser 101 ser			