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Significance of matrix metalloproteinase-1 and -3 gene polymorphisms and their expression in normal and neoplastic endometrium

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ABSTRACT

Background: Matrix metalloproteinases (MMPs) play a fundamental role in tissue degradation or remodeling and involved in all stages of tumor progression. However, the role of the MMPs polymorphisms with endometrial carcinoma has not been fully examined in Egyptian patients. Therefore, we planned this study to evaluate associations of the MMP-1 (− 1607 1G/2G) and MMP-3 (− 1171 5A/6A) polymorphisms and their immune reactive protein combinations with endometrial carcinoma susceptibility and prognosis in Egyptian patients.
Methods: Paraffin-embedded tissue samples from 40 women with endometrial carcinoma and 30 controls were immunohistochemically stained for MMP-1and MMP-3 expression. Tissue MMPs levels were analyzed by ELISA technique, and tissue samples were genotyped for MMP-1 and MMp-3 gene polymorphisms by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).
Results: we recorded a significant increase of MMP-1 levels in endometrial carcinoma patients more than controls, to be more increased with advanced stages and grades of the carcinoma. Whereas, the MMP-3 tissue levels showed non-significant changes among patients and controls and even among different stages and grades of cancer.

Patients with endometrial carcinoma exhibited a higher distribution of MMP-11G/2G or 2G/2G genotypes compared with controls. Tumors containing the 2G allele expressed MMP-1 protein more frequently than those of 1G/1G genotype. The overall genotype and alleles distribution of the MMP-3 polymorphism in patients was not different from that of controls. The haplotype 2G-6A was associated with a significantly increased risk of endometrial carcinoma as compared with the 1G-5A haplotype.
Conclusion: The MMP-1 (− 1G/2G) SNP and MMP 2G/6A haplotype may modify susceptibility and are associated with a higher risk of endometrial carcinoma. Otherwise, the MMP-3 (5A/6A) promoter SNP is unlikely to be associated with endometrial carcinoma in the Egyptian population.

1. Introduction

Normal cells may undergo genetic changes that yield to phenotypic changes and malignancy development which is a complicated, multistage process. Cancer cells having the ability to invade and metastasize the distant organs (Weinberg, 1989). The tumorigenesis is a multifactorial process, many factors control tumor development (Al-Zhoughbi et al., 2014). The interaction between cancer cells and the microenvironment is critical to tumorigenesis process (Al-Zhoughbi et al., 2014). The Metalloproteinase (MMP) family constitutes over than 25 proteolytic enzymes that are responsible for degradation of the extracellular matrix (ECM), including the basement membrane (Parsons et al., 1997). MMPs are further classified into 6 types on the basis of structure and their specificity to specified substrates (Ninomiya et al., 2004). Overexpression of MMP-1 (collagenase) is highly correlated to the tissue-destructive processes pathogenesis in some diseases (Tanioka et al., 2003). Increase the level of MMP-1 expression can be seen in multiple disorders and it has been detected in endometrial lesions (Hudelist et al., 2005a, 2005b). MMP-3 (*stromelysins*, STMY1) is a vital member of the MMP family and degrades several collagen tissue types (Nagase and Okada, 1997). Pro-MMP-3 and MMP-3 are critical in the remodeling process of connective tissue and crucial in the ECM components turnover (Lesauskaite et al., 2008). High expression of MMP-3 in some tumors is implicated in tumor angiogenesis, invasion, and

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metastasis (Raffetto and Khalil, 2008; Gaide Chevronnay et al., 2012; Velinov et al., 2010). The MMP-1 and MMP-3 genes are located on the negative strand of chromosome 11, and functional polymorphisms that affect their transcription levels have been identified for each (Ye et al., 1995; Ye et al., 1996; Rutter et al., 1998; Jormsjo et al., 2001). The MMP-1 1G/2G polymorphism, resulting from the insertion/deletion of a guanosine at nucleotide position 1607, has been reported to be associated with a variety of malignancies (Zhang et al., 2013). The 2G polymorphism creates a binding site (5′-GGA-3′) for the ETS transcription factor, influencing its transcriptional activity (Tower et al., 2002). Promoter containing 2G allele displays a significantly higher transcriptional activity than 1G promoters (Ye et al., 1996). The 2G allele of the MMP-1 promoter polymorphism is relatively common and has a frequency of a little < 50% of the general population (Ye et al., 1996). Association studies have been done to determine whether the MMP-1 genotype affects the risk of different types of cancers (Zhu et al., 2001; Nishioka et al., 2000; Ghilardi et al., 2001; Hinoda et al., 2002). The MMP-3 gene is located adjacent to the telomere side of the MMP-1 gene on 11q22 (Ye et al., 1995). A naturally occurring and common polymorphism (rs3025058) which affects the MMP-3 promoter at a position − 1171, where either 5 or 6 consecutive adenines (5A/6A) affect the binding of transcription factor and alter MMP-3 promoter activity (Ye et al., 1996). The upcoming evidence suggests a complicated role for TIMPs through tumor progression with a more complicated role in the regulation of ECM degradation (Chaudhary et al., 2016; Brown and Murray, 2015; Yadav et al., 2014). Tumor transgression, angiogenesis and tumor metastasis impose of ECM degradation and remodeling; therefore, it is clear that the expression of MMPs is associated with the invasion and metastasis of diverse malignancies. The relationship between the MMP-3 5A/6Apolymorphism and susceptibility to cancer remains ambiguous (Hirata et al., 2004). Previous studies on MMPs SNPs and endometrial adenocarcinoma are very scanty, few studies have evaluated a single MMP-1 single nucleotide polymorphism (SNP) and reported inconsistent results (Nishioka et al., 2000; Beeghly-Fadiel et al., 2009). However, till now no published studies concerning the role of the MMPs polymorphisms in Egyptian patients with endometrial cancer have been reported as yet. Therefore, we aimed to evaluate associations of the MMP-1 (− 1607 1G/2G) and MMP-3 (− 1171 5A/6A) polymorphisms and altered protein amounts in normal and malignant cells, moreover to examine the effect of MMP-1 and MMP-3 immune reactive protein combinations on the susceptibility prognosis of endometrial carcinoma in Egyptian patients.

2. Subjects and methods

This study was carried out at Medical Biochemistry and Pathology departments, Faculty of Medicine, Zagazig University, Egypt from May 2015 to Sep 2016. A total 70 subjects were included in the study, they were selected from the Database of Pathology Department, Faculty of medicine, Zagazig University. The study was approved by the ethical committee of the Zagazig University.

40 patients with histologically confirmed endometrial carcinoma of paraffin-embedded tumor tissue sections were included in the study, their mean age ± SD was 48.25 ± 11.69 years (range 26–83 years). The extra-fascial hysterectomy, bilateral salpingo-oophorectomy, and pelvic lymphadenectomy were the first line treatment in the all patients. For more confirmation, all tissues samples were reviewed by two pathologists. None of the 40 patients have received chemotherapy or radiotherapy before the surgical intervention.

The International Federation of Gynecology and Obstetrics (FIGO) guidelines were applied to determine the endometrial adenocarcinoma histological stage and grade to be considered as markers of the disease prognosis in the current study. (Benedet et al., 2000).

The frequency and percentage of different stages and grades of the 40 endometrial carcinomas are listed in Table 1. Regarding the FIGO criteria, there were 10 patients (25%) with stage I, 12 (30%) patients with stage II, 11 (27.5%) stage III patients, and 7 (17.5%) patients with stage IV of endometrial carcinoma. Of all 40 tumors, there were 17 (42.5%) grade 1, 13 (32.5%) moderately differentiated of grade 2, and 10 (25%) poorly differentiated of grade 3.

Table 1
Frequency and percentage of different stages and grades according to FIGO classification of endometrial adenocarcinoma patients.

Endometrial adenocarcinoma	Frequency (*n* %)
Stages total *n* = 40	
Stage I	*n* = 10, 25%
Stage II	*n* = 12, 30%
Stage III	*n* = 11, 27.5%
Stage IV	*n* = 7, 17.5%
Grades	
Grade 1	(*n* = 17, 42.5%)
Grade 2	(*n* = 13, 32.5%)
Grade 3	(*n* = 10, 25%)

30 randomly selected control subjects were included in the study. They had undergone hysterectomy for benign conditions and all had exclusion criteria from endometrial carcinoma. Their mean age ± SD was 45.26 ± 11.12 years (26–63 years). Hematoxylin and eosin-stained section of each tissue blocks were applied to confirm the diagnosis and ensure the absence of tumor prior the DNA extraction.

All tissue samples from patients and controls were examined for tissue MMP-1 and MMP-3 levels using ELISA technique, Immunohistochemical staining was done for determination the MMP-1 and MMP-3 staining and DNA extraction was performed to examine the MMP-1 (− 1607 1G/2G) and MMP-3 (− 1171 5A/6A) polymorphisms with endometrial adenocarcinoma susceptibility and prognosis.

2.1. Tissue MMP-1 and MMP-3 levels

A sandwich enzyme-linked immunosorbent assay (ELISA) (R & D Systems, Catalog #DMP 300) was implemented for Tissue MMP1 and MMP3 estimation. The paraffin-embedded tissues which delivered from Pathology department were carved with a surgical blade into 1 mm pieces, between 25 and 75 mg of each paraffin embedded tissue block was transferred to a micro tube for homogenization with cell lysis buffer. Centrifugation of the homogenized sample was performed at10.000 rpm for 10 min and then the supernatant was collected for the MMPs ELISA estimation.

2.2. Extraction of DNA

Dissection was performed to the tissue and prepared for DNA extraction after deparaffinization. Genomic DNA was then extracted from the deparaffinized tissue using the conventional phenol/chloroform method following the proteinase K digestion (Cawkwell, 2002). An equal volume of Tris-saturated phenol (pH = 8) was added to the tubes and centrifuged at 12000 for 3 min. 300 µl was transferred to an equal volume of phenol –chloroform mixture (1:1) and after centrifugation 2.5 volume of ethanol was added for DNA precipitation.

The DNA pellet was gently washed with 70% ethanol and then the pellet was dried and dissolved in 70 µl distilled water to be ready for PCR run.

2.3. Genotyping

Identifying the MMP-1 (− 1607 1G/2G) and MMP-3 (− 1171 5A/6A) polymorphisms were performed using polymerase chain reaction/restriction fragment length polymorphism ((PCR/RFLP) method described by Zhu et al., 2001;Gnasso et al., 2000).

The PCR primers used for amplifying the MMP-1 polymorphism were: the sense primer 5′-TGACTTTTAAAACATAGTCTATGTTCA-3′-