

A Single Nucleotide Polymorphism in the Matrix Metalloproteinase-1 Promoter in Endometrial Carcinomas

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Recent studies demonstrated that a single guanine insertion polymorphism in a matrix metalloproteinase-1 promoter created an Ets binding site and affected the elevation of the transcriptional level of matrix metalloproteinase-1 (MMP-1). Furthermore, in tumor cell lines derived from melanoma and breast cancer, the incidence of the 2G/2G genotype was significantly higher than that in the normal population. To evaluate the contribution of this polymorphism in endometrial carcinomas, we genotyped 100 endometrial carcinomas and then analyzed immunoeexpression of MMP-1 in these carcinomas. We found that endometrial carcinoma patients showed a significantly higher rate of 1G/2G or 2G/2G genotype than control individuals, and that tumors containing the 2G allele(a) expressed MMP-1 protein more frequently than those with 1G/1G genotype. Therefore, the single nucleotide polymorphism at the MMP-1 promoter affected the expression level of the MMP-1 protein, which may result in the association with more aggressive character in endometrial carcinoma. Our result suggests that the presence of 2G polymorphism at the MMP-1 promoter may be one of the risk factors for the development and/or progression of endometrial carcinoma.

Key words: Matrix metalloproteinase-1 — Single nucleotide polymorphism — Human endometrial carcinoma

Endometrial carcinoma is a common gynecologic malignancy. The number of deaths from endometrial carcinoma has been increasing from year to year,¹ yet little is known about the molecular events underlying tumor development and/or progression. Clinical study of gynecological cancers demonstrated that endometrial carcinoma is frequently accompanied with extensive invasion of uterine muscle and/or distant metastasis. Despite recent improvements in early diagnosis, surgical techniques, and chemotherapy, about one-third of the patients who undergo operative resections will die within 10 years due to recurrent disease or metastases resistant to conventional therapies.² Spread of malignant tumors is a multi-step process and many of the steps of tumor invasion require degradation or breakdown of the extracellular matrix and connective tissue surrounding tumor cells.^{3,4} Matrix metalloproteinases (MMPs) are a class of structurally related enzymes that function in the degradation of the extracellular matrix proteins that constitute connective tissue.⁵ MMPs are essential for tumor cells to penetrate the basement membrane and colonize distant sites. Among MMPs, MMP-1 is the most ubiquitously expressed inter-

stitial collagenase, thereby claiming a prominent role in collagen degradation.⁶ Clinical research has demonstrated the presence of MMP-1 in cancer cells and has shown that MMP-1 expression is associated with poor prognosis.^{7,8}

Recently, Rutter *et al.*⁹ reported a single nucleotide polymorphism (SNP) at -1607 bp in the MMP-1 promoter, where a guanine (G) insertion creates an Ets binding site, 5'-GGA-3', flanking an AP-1 site. This 2G allele, G insertion polymorphism, has significantly higher transcriptional activity in normal and melanoma cells than 1G allele. Frequent 2G homozygote polymorphism has been detected in tumor cell lines derived from melanoma and breast cancer compared to the normal population. In fact, a correlation between the expression of MMP-1 and single nucleotide polymorphism in the promoter region has been found recently in ovarian carcinomas.¹⁰ In order to clarify the contribution of the single nucleotide polymorphism to the development of endometrial carcinoma, we performed nucleotide sequence analysis of the MMP-1 promoter region in endometrial carcinomas and an immunohistochemical study of MMP-1.

MATERIALS AND METHODS

DNA preparation Materials used in this study were obtained during the course of surgical treatments at Sapporo Medical University. We examined 44 fresh frozen

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Abbreviations: MMP-1, matrix metalloproteinase-1; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; LOH, loss of heterozygosity.

samples of normal and tumor tissue in patients with endometrial carcinoma. In addition, 56 paraffin-embedded samples of normal tissues and cancerous lesions from patients with endometrial carcinoma were also examined. DNAs were extracted from fresh frozen samples according to methods described elsewhere,¹¹⁾ and from paraffin-embedded samples by using a DEXPAT kit (TaKaRa, Otsu) system according to the manufacturer's directions. Histological diagnosis of each tumor was done according to the WHO classification,¹²⁾ and the clinical stage was determined according to the International Federation of Gynecology and Obstetrics.¹³⁾ Control samples consisted of DNA extracted from whole blood collected from 150 healthy women.

Nucleotide sequence analysis To search for 1G/2G polymorphism in the MMP-1 promoter, PCR amplification was performed with a pair of primers, M-F (5'-ACATGTTATGCCACTTAGAT-3') and M-R (5'-TCCCCTTATGGATTCTTGTT-3'), followed by nucleotide sequence analysis. In this study, each 20 µl reaction mixture contained 1× PCR buffer (TaKaRa), 25 pmol of each primer, 50 ng of genomic DNA or 2 µl of crude extract, 1.25 mM dNTP and 0.25 units of *Taq* DNA polymerase (TaKaRa). Reaction mixtures were heated to 94°C for 2 min, and then cycled 35 times; each cycle consisted of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and strand elongation at 72°C for 30 s. The PCR products were gel-purified, and the nucleotide sequences were determined by using a Dye terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA) with each of PCR primers as a sequence primer, on an Applied Biosystems model 377 DNA sequencer (Perkin-Elmer Cetus, Norwalk, CT).

Immunohistochemical analysis Immunostaining for MMP-1 was performed with an MMP-1 monoclonal antibody (Fuji Chemical Ind., Ltd., Takaoka City), as described previously with some modifications.⁸⁾ We assessed the level of MMP-1 expression in tumor cells by comparison with that in normal tissue on the same paraffin-embedded slice as a control. Fifty cases of endometrial carcinomas were categorized into the following three groups as regards MMP-1 immunostaining; negative, weaker or similar staining intensity as compared with the corresponding normal tissue; moderate or somewhat greater staining as compared with the corresponding normal tissue (up to 3×); strong or marked staining (more than three times the corresponding normal tissue).

RESULTS

In order to search for 1G/2G polymorphism at -1607 bp in the MMP-1 promoter, we performed PCR-sequence analysis of normal tissue DNAs from 100 patients and 150 control individuals (Fig. 1, A-C). Genotyping analysis

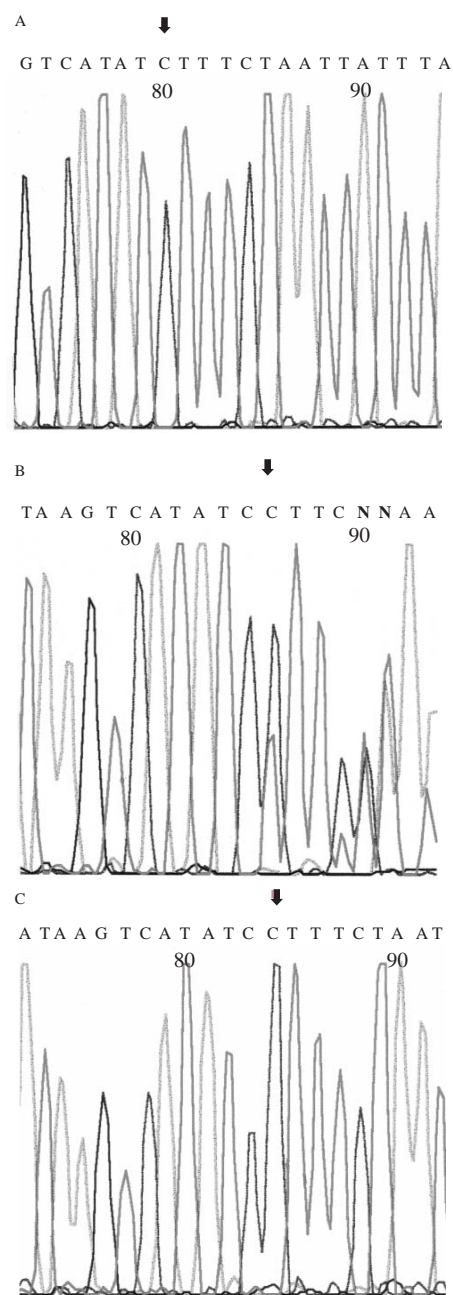


Fig. 1. Nucleotide sequence of MMP-1 promoter region in anti-sense direction. Arrows indicate genotypes of 1G/1G (A), 1G/2G (B), and 2G/2G (C) at -1607 bp.

revealed that the incidence of —/2G genotypes was significantly higher in patients with endometrial carcinomas than in control individuals (Table I). To clarify the possible role of loss of heterozygosity (LOH) at this polymorphism, we conducted PCR-sequence analysis of tumor DNAs in all cases with 1G/2G genotype. We found that

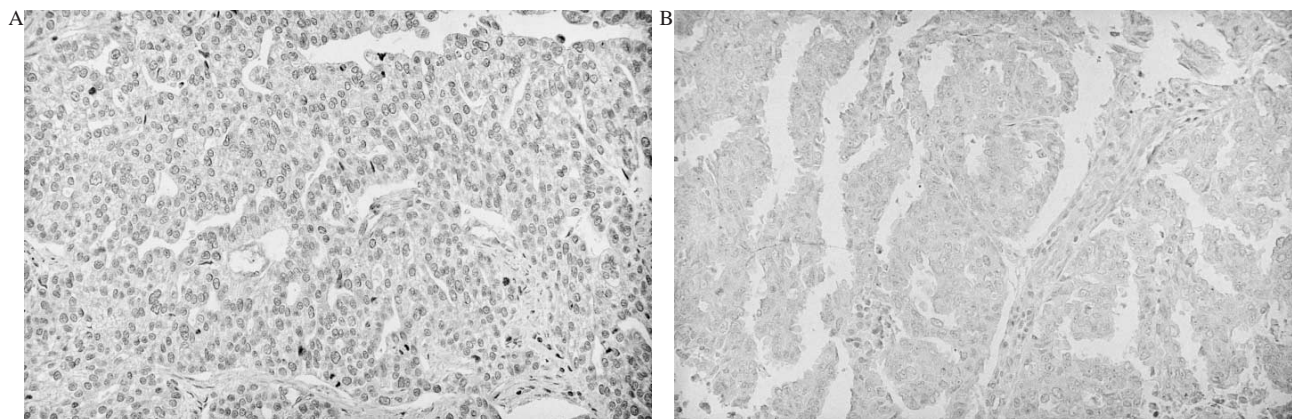


Fig. 2. Representative presentation of immunohistochemical stainings by anti-MMP-1 antibody. The cancerous lesion was strongly stained in T-17 (2G/2G genotype) (A), whereas MMP-1 expression was not detected in T-5 (1G/1G genotype) (B).

Table I. The Numbers of Each MMP-1 Promoter Genotype in Normal Controls and Patients with Endometrial Carcinomas

| | 1G/1G | 1G/2G | 2G/2G |
|---------------------------|-------|-------|-------|
| Controls (<i>n</i> =150) | 30 | 56 | 64 |
| Patients (<i>n</i> =100) | 9 | 41 | 50 |

The frequency of —/2G (1G/2G or 2G/2G) genotypes was significantly higher in the patients than in normal controls. χ^2 ; *P*=0.019.

Table II. Genotypes at the Promoter Region and Immunohistochemical Study of MMP-1 in Endometrial Carcinomas

| | Negative | Positive | |
|-----------------------|----------|----------|--------|
| | | moderate | strong |
| 1G/1G (<i>n</i> =5) | 3 | 2 | 0 |
| 1G/2G (<i>n</i> =22) | 3 | 9 | 10 |
| 2G/2G (<i>n</i> =23) | 5 | 6 | 12 |

Patients with 2G allele(a) were more frequently stained with MMP-1 antibody than those with 1G/1G genotype (82.2% of patients with —/2G genotypes were positively stained compared to 40.0% of those with 1G/1G genotype; statistically not significant).

none of these cases showed LOH at this locus. Furthermore, to investigate whether the genotype in SNP is associated with MMP-1 expression in cancer cells, we performed immunohistochemical analysis of MMP-1 in 50 endometrial carcinomas, for which tissue samples were available (Fig. 2). Combining the results of genotyping and immunostaining, patients who possess 2G allele were more frequently stained with MMP-1 antibody compared with patients of 1G/1G genotype (Table II). To characterize the spectrum of 2G polymorphism in endometrial

carcinomas, we evaluated the correlation between the genotypes and clinicopathological parameters, such as onset age, prognosis, histology, and stage. However, no significant association between the polymorphism and the clinicopathologic factors was found.

DISCUSSION

In this study, we genotyped 100 patients with endometrial carcinomas and 150 normal individuals and found that the proportion of 1G/2G, 2G/2G genotypes in the patients was significantly higher than that in a normal population. We also found that patients with the 2G allele(a) more frequently expressed MMP-1 than those with 1G/1G genotype. The frequency of MMP-1 expression in tumors with 1G/2G genotype was similar to that in tumors with 2G/2G type. As none of the cases showed LOH, 2G polymorphism in the MMP-1 promoter appears to affect the expression of MMP-1 dominantly. Our results suggest that tumors in patients with the 2G allele may exhibit more invasive behavior due to high levels of MMP-1 expression. Our results also suggest that the presence of 2G polymorphism at the MMP-1 promoter may be a risk factor for the development and/or progression of endometrial carcinoma; this putative association between genotype and clinical behavior, particularly prognosis, should be confirmed in a larger group of patients.

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