Correlation between Expression of the Matrix Metalloproteinase-1 Gene in Ovarian Cancers and an Insertion/Deletion Polymorphism in Its Promoter Region

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Abstract

Matrix metalloproteinases (MMPs), a family of closely related enzymes that degrade the extracellular matrix, are likely to be involved in invasion and metastasis of tumor cells. A guanine (G) insertion/deletion polymorphism within the promoter region of MMP-1 influences the transcription of this gene; i.e., the 2G (insertion-type) promoter possesses greater transcriptional activity than the 1G (deletion-type) promoter. To investigate whether this feature contributes to cancer development and/or progression, we genotyped 163 ovarian cancer patients for the polymorphism and then analyzed levels of expression of the MMP-1 gene in their tumors. The proportion of patients who were either heterozygotes or homozygotes for the 2G allele was significantly higher than that observed among 150 individuals without cancer (P = 0.0028). Moreover, the levels of MMP-1 expression in cancer tissues among the patients carrying 2G alleles were elevated significantly in comparison with 1G homozygotes (P = 0.0038).

Introduction

Ovarian cancer, one of the most common gynecological malignancies, has an aggressive phenotype and a relatively poor prognosis; peritoneal dissemination and/or retroperitoneal lymph node metastases are found in two-thirds of patients at the time of diagnosis (1). To improve prognosis of this disease, it is vital to clarify the molecular mechanisms involved in the invasion and metastasis of ovarian cancers.

Tumors spread by way of a multistep process, in which degradation of extracellular matrix and basement membrane barriers is a key feature (2–4). MMPs\(^\text{1}\) comprise a family of at least 16 proteolytic enzymes that degrade extracellular matrix in a substrate-specific manner, and they are thought to have important roles in tumor invasion and metastasis (2, 5–7). Among the MMPs, MMP-1 (interstitial collagens) degrades fibrillar collagens, the most abundant class of extracellular matrix proteins in interstitial connective tissue. Overexpression of MMP-1 has been demonstrated in tumor tissues and cell lines (8–10), and patients whose tumors express a high level of MMP-1 production may enhance development and/or rapid progression of ovarian cancers.

Materials and Methods

Samples. Whole blood and tumor specimens were obtained from 163 patients who had undergone surgery for ovarian cancer. All patients gave informed consent prior to collection of samples, according to institutional guidelines. Tumor specimens had been collected at the time of surgery, snap-frozen, and stored at −80°C after being histologically confirmed. Borderline tumors were excluded a priori from our study. Genomic DNA was prepared by digestion with proteinase K and phenol-chloroform extraction. Control samples consisted of DNA extracted from whole blood collected from 150 healthy women.

Radiolabeled-PCR Assay. Analysis of the insertion/deletion polymorphism in the MMP-1 promoter was performed as described elsewhere (13), with some modifications. Primers for PCR amplification were: sense, 5'-GGATATGGCCTAGTAGG-3'; and antisense, 5'-CTCTCCCCTTAGGATGCC-3'. The antisense primer (50 pmol) was end-labeled with \(^{32}\)PdATP. PCR amplification was performed in a 10-μl reaction volume containing 20 ng of genomic DNA, 5 pmol of each primer, 1.25 mM dNTPs, 67 mM Tris-HCl (pH 8.8), 16.6 mM (NH\(_4\))\(_2\)SO\(_4\), 10 mM -mercaptopethanol, 6 μM EDTA, 6.7 mM MgCl\(_2\), 10% DMSO, and 0.25 units of EX Taq DNA polymerase (TaKaRa, Tokyo, Japan). PCR conditions were as follows: 2 min at 94°C; 25 cycles of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C; and then 5 min at 72°C. Following amplification, an equal volume of stop buffer was added, and the products were denatured for 5 min at 80°C. Samples (2.5 μl each) were loaded onto a 7% denaturing polyacrylamide gel. After electrophoresis, the gels were dried and exposed to X-ray films. We invoked a χ\(^2\) test to examine associations between genotypes of the insertion/deletion polymorphism and the presence of cancer (StatView software).

RNA Extraction and Semiquantitative RT-PCR. Total RNA was extracted from the 29 tumor tissue samples, for which genotypes of the insertion/deletion polymorphism in corresponding normal tissues were determined (9 1G/1G homozygotes, 10 1G/2G heterozygotes, and 10 2G/2G homozygotes), using TRIzol reagent (Life Technologies, Inc.) according to the manufacturer’s protocol. For RT-PCR, 10 μg of total RNA were reverse-transcribed to the first strand using random hexamers as primers.

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than that in controls (80%; \( P \text{b} \)). Table 1 shows the genotypes in ovarian cancers (89%) was higher than that in controls (80%; \( P = 0.028 \)). To further investigate whether this insertion/deletion polymorphism influences expression of the \( MMP-1 \) gene in ovarian cancers, we performed semiquantitative RT-PCR experiments using tumor tissues with genotypes that had been determined (Fig. 2A). In tumors carrying \( 2G \) alleles (\( 1G/2G \) homozygotes), the median expression levels of \( MMP-1 \) were >7 times the median level of tumors having no \( 2G \) alleles (Fig. 2B). The intensities of the \( MMP-1 \) bands calculated as a ratio against intensities of corresponding \( \beta-2 \) microglobulin bands were 1.5 versus 0.2, respectively (Mann-Whitney \( U \) test, \( P = 0.0038 \)).

Discussion

The insertion or deletion on the \( MMP-1 \) promoter region of the \( MMP-1 \) gene, which determines the presence or absence of an Ets binding site, affects the transcriptional level of \( MMP-1 \) in normal fibroblasts and in melanoma cells. Furthermore, in tumor cell lines, the frequency of homozygotes for the insertion type was significantly higher than that in normal population (13). On the basis of those findings, Rutter et al. (13) hypothesized that the insertion/deletion polymorphism might influence the transcriptional responsiveness of the \( MMP-1 \) promoter in clinical settings because excessive production of \( MMP-1 \) is a major contributor to the stromal degradation involved in tumor invasion (11, 12). The high frequency of \( 2G \) homozygotes in tumor cell lines has been taken to imply an increase in invasive behavior due to high levels of \( MMP-1 \) expression.

In the work reported here, we investigated the frequencies of the different insertion/deletion alleles in blood samples from patients with ovarian cancer and examined expression of \( MMP-1 \) in some of their tumors. We used genomic DNAs with genotypes for the insertion/deletion polymorphism that had been determined by direct sequencing (bottom).

Results

The insertion/deletion polymorphism in the \( MMP-1 \) promoter yielded PCR products of 148 bp (deletion type) and 149 bp (insertion type; Fig. 1). When we amplified DNAs from blood samples of 163 ovarian cancer patients and 150 control individuals, we found a significant difference in allelic frequencies at this polymorphic site between patients and controls (Table 1). The frequency of heterozygotes and homozygotes for the \( 2G \) allele (\( 1G/2G \) and \( 2G/2G \)) was significantly higher among women with ovarian cancers (89%) than that in individuals without cancer (80%; \( \chi^2 = 4.827, P = 0.028 \)), indicating that individuals who carry even one \( 2G \) allele are more susceptible to ovarian cancer than women with a constitutional \( 1G/1G \) genotype.

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tumors. We demonstrated that 1G/2G and 2G/2G genotypes were significantly more frequent in patients with ovarian cancer than they were in normal controls. Although a high frequency of 2G homozygotes might have been anticipated in view of the earlier report concerning tumor cell lines (13), we also found that heterozygosity for the 2G allele, not only homozygosity, seemed to increase susceptibility to ovarian cancer.

Subsequent analysis of MMP-1 expression in ovarian cancers of all three genotypes revealed that expression of this gene was markedly higher in tumors having 1G/2G and 2G/2G genotypes than in tumors with the homozygotic 1G genotype. We examined the tumors of the 10 constitutional heterozygotes for LOH at the MMP-1 locus to compare the effect of LOH on expression of MMP-1 in normal and tumor tissues. Because only one of those cases examined revealed LOH, we were unable to obtain any useful information. However, in the tumor that had lost the 2G allele (corresponding to Lane 18 in Fig. 2A), the level of MMP-1 expression was low. Histopathological type of ovarian cancers did not correlate to the level of MMP-1 expression.

Our overall results imply that the genotype for the insertion/deletion polymorphism in its promoter sequence influences the expression of the MMP-1 gene in ovarian cancer tissues. Because the 2G type of promoter possesses a binding site for the Ets transcription factor, such binding is likely to lead to higher transcription of MMP-1. An excess of MMP-1 production may contribute to enhance degradation of the extracellular matrix and, thereby, promote invasion and metastasis of tumor cells. This scenario could partially explain the generally aggressive character of ovarian tumors. Because the expression of MMP-1 are heterogeneous in each class (Fig. 2), other factors/sequences will be also contribute to this activity.

We have suggested here that a 2G genotype of the MMP-1 promoter might represent a risk factor for development and progression of ovarian cancer. A clinical study should be undertaken in larger populations to investigate the correlation between alleles of this polymorphic site and prognosis of individual ovarian cancers.

References
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