Invasiveness of Cutaneous Malignant Melanoma Is Influenced by Matrix Metalloproteinase 1 Gene Polymorphism

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Abstract

The matrix metalloproteinases (MMPs) are implicated in connective tissue destruction during cancer invasion and metastasis. A naturally occurring variant arising from the insertion or deletion of a guanine in the promoter of the MMP-1 gene has recently been reported and shown to influence its transcriptional activity in melanoma cells. In this study, MMP-1 genotype was determined in 139 Caucasian patients with cutaneous malignant melanoma. The insertion allele was associated with deep invasive, and therefore poorer-prognosis, primary tumors (34%) of patients with vertical growth phase tumor were homozygous for the insertion allele compared with 17% of patients with horizontal growth phase tumor (P = 0.0333; odds ratio = 2.51). These data suggest that the invasiveness of cutaneous malignant melanoma is influenced by variation in the MMP-1 gene promoter that affects MMP-1 expression.

Introduction

Degradation of the extracellular matrix and basement membrane barriers is a key process in tumor invasion and metastasis. There is strong evidence indicating that the MMPs, which possess proteolytic activities against extracellular matrix and basement membrane proteins, play an important role in this process and therefore facilitate tumor invasion and spread (1, 2). Recently, a naturally occurring sequence variation in the human MMP-1 gene promoter was reported (3). This genetic variation arises from insertion or deletion of a G at position 1607 relative to the transcriptional start site; consequently one allele (insertion) has two 2Gs, whereas the other allele (deletion) has only 1G at this position. The insertion creates the core sequence (GG-TGA-3) of a binding site for the Ets transcription factors, and it was demonstrated in vitro that the 2G allele had a higher transcriptional activity in melanoma cells (3). CMM is the most serious cutaneous malignancy. Relatively little is known of the genetic factors underlying susceptibility to and prognosis in sporadic CMM, although polymorphisms in several genes have been implicated (4–6). In this study, we sought to determine whether this MMP-1 gene polymorphism is associated with susceptibility to CMM in British Caucasian subjects, and whether this polymorphism influences CMM prognosis, i.e., is associated with known prognostic features of CMM, particularly those associated with tumor invasiveness, growth, recurrence, or metastasis.

Subjects and Methods

Subjects

Patients. Formalin-fixed and paraffin-embedded tissue blocks from 139 CMM patients (presenting in 1986–1993) were retrieved from the files of the Histopathology Department, Southampton General Hospital (Southampton, United Kingdom). All specimens were re-reviewed by two histopathologists (A. C. B. and J. M. T.) and the original diagnoses of CMM confirmed.

Tumor Histopathology Data. Histopathological prognostic features of each case were assessed as defined in the literature and used in previous studies (7, 8). Radial growth phase CMMs were defined as those limited in extent to the epidermis (melanoma in situ) or showing early invasion of the upper dermis but with dermal nests of melanocytes no larger than those at the dermoeidermal junction and containing no mitotic figures. Vertical growth phase CMM showed expansive growth within the dermis, evidenced by nests of neoplastic melanocytes that were larger than those at the dermoeidermal junction or by the presence of mitotic figures within dermally located melanocytes (8). For vertical growth phase CMM, the mitotic count/mm² of tumor was assessed as nil, 1–6, or >6, and the number of tumor-infiltrating lymphocytes was evaluated as absent, nonbrisk/focal, or brisk (8). The presence of tumor regression, defined as segmental tumor loss, was also recorded.

Clinical Follow-up Data. The following variables were recorded for each patient, subject to availability of clinical data: (a) gender; (b) age; (c) site of CMM: (d) length of clinical follow-up; (e) presence of recurrent or metastatic tumor; (f) disease-free survival; and (g) overall survival time. The clinicopathological stage of each patient at initial presentation for whom full data were available was calculated using the Tumor-Node-Metastasis system (9).

Controls. Controls consisted of stored DNA samples derived from 142 cadaveric and noncadaveric solid organ and bone marrow donors. All patients and donors were Caucasian.

Preparation of DNA Samples

DNA was extracted from Formalin-fixed, paraffin-embedded tissue blocks from CMM patients as described previously (7, 10). Briefly, two to five 20-μm sections were cut from each tissue block and dewaxed in xylene (Merck, Ltd., Poole, United Kingdom) and xylene-ethanol washes. DNA was extracted from the resulting cellular material by proteinase-K digestion. Control DNA samples were originally prepared from peripheral blood by standard salt-precipitation protocols (11).

Determination of Genotypes

A recently published method was used to determine the genotypes of the subjects (12). Briefly, PCR was carried out in a total volume of 25 μl containing 50 ng of genomic DNA; 10 pmol of the forward and reverse primers (5’-TCGAGAAGTCTTCCTCATT-3’ and 5’-TCGAGGATTGAGGATTTTGAG-3’; respectively); 200 μm each dATP, dCTP, dGTP, and dTTP; 20 μM Tris-HCl (pH 8.4); 50 mM KCl; 0.05% (v/v) W1 (Life Technologies, Inc.); 1.5 mM MgCl₂; and 1 unit Taq polymerase (Life Technologies, Inc.). The solution was overlaid with 25 μl of liquid paraffin and incubated for 15 min at 94°C, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The PCR products were digested with HpaII (Promega) and 10 μl of the digest was subject to electrophoresis in a 2% agarose gel. DNA samples were digested with HpaII to a length of approximately 250 bp. The resulting bands were visualized under ultraviolet light. A recently published method was used to determine the genotypes of the subjects (12). Briefly, PCR was carried out in a total volume of 25 μl containing 50 ng of genomic DNA; 10 pmol of the forward and reverse primers (5’-TCGAGAAGTCTTCCTCATT-3’ and 5’-TCGAGGATTGAGGATTTTGAG-3’; respectively); 200 μm each dATP, dCTP, dGTP, and dTTP; 20 μM Tris-HCl (pH 8.4); 50 mM KCl; 0.05% (v/v) W1 (Life Technologies, Inc.); 1.5 mM MgCl₂; and 1 unit Taq polymerase (Life Technologies, Inc.). The solution was overlaid with 25 μl of liquid paraffin and incubated for 15 min at 94°C, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The PCR products were digested with HpaII (Promega) and 10 μl of the digest was subject to electrophoresis in a 2% agarose gel. DNA samples were digested with HpaII to a length of approximately 250 bp. The resulting bands were visualized under ultraviolet light. A recently published method was used to determine the genotypes of the subjects (12). Briefly, PCR was carried out in a total volume of 25 μl containing 50 ng of genomic DNA; 10 pmol of the forward and reverse primers (5’-TCGAGAAGTCTTCCTCATT-3’ and 5’-TCGAGGATTGAGGATTTTGAG-3’; respectively); 200 μm each dATP, dCTP, dGTP, and dTTP; 20 μM Tris-HCl (pH 8.4); 50 mM KCl; 0.05% (v/v) W1 (Life Technologies, Inc.); 1.5 mM MgCl₂; and 1 unit Taq polymerase (Life Technologies, Inc.). The solution was overlaid with 25 μl of liquid paraffin and incubated for 15 min at 94°C, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The PCR products were digested with HpaII (Promega) and 10 μl of the digest was subject to electrophoresis in a 2% agarose gel. DNA samples were digested with HpaII to a length of approximately 250 bp. The resulting bands were visualized under ultraviolet light.
1 min at 95°C, and then by 35 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C. A 15-μl aliquot of PCR products was mixed with a 5-μl solution containing 2 μl of 10× NEBuffer 2 (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl₂, and 1 mM DTT (pH 7.9)), 0.2 μl of BSA (10 mg/ml), 0.3 μl Xmn I (20 units/ml), and 2.5 μl of sterile deionized H₂O. The 5-μl aliquot of the digests was mixed with 2 μl of loading buffer and electrophoresed on a 10% horizontal nondenaturing polyacrylamide gel at 150 V for 2.5 h. The gel was then stained with Vistra Green (Amersham) and scanned with a fluorimager (FI595; Molecular Dynamics, Sunnyvale, CA).

**Results**

**MMP-1 Genotype and Susceptibility of Melanoma.** **MMP-1** genotype according to insertion (2G allele) or deletion (1G allele) of a G at position −1607 (3) was determined in 139 patients and 142 healthy Caucasian subjects. No statistically significant difference in genotype and allele frequencies was detected between the melanoma patients and the healthy subjects, with the frequency of the 2G allele being 53% in patients and 47% in healthy subjects (Table 1). The genotype frequencies in both samples were consistent with Hardy-Weinberg equilibrium distribution.

The allele frequencies in the above healthy Caucasian subjects were significantly different from reported allele frequencies in healthy Japanese (13; P = 0.0465; Table 1).

**MMP-1 Genotype and Invasiveness of Melanoma.** In the CMM patients, there was an association between the 2G allele and deep invasive tumors; 34% of patients with vertical growth phase lesions were homozygous for the 2G allele compared with 17% of patients with horizontal growth phase lesions (P = 0.0333; Table 2).

Analysis of disease-free survival was carried out in 99 subjects who had a complete recovery from surgery and were then followed up clinically. There was a trend toward decreased disease-free survival in patients carrying the 2G allele and stage at presentation (I versus II/III), Breslow depth (greater than or less than 1.5 mm) and mitotic count in vertical growth phase CMM, presence of tumor infiltrating lymphocytes, and presence or absence of relapse.

**Discussion**

CMM is a serious and potentially fatal condition, with the most important environmental risk factor being UV light exposure and ~5–10% of all cases being familial (14). In nonfamilial CMM cases, both susceptibility to and prognosis in this malignancy have been shown to be influenced by human leukocyte antigen DQB1 polymorphisms (7) or DQB1-associated haplotypes (15), although other nonimmunogenetic factors have also been implicated (4–6). The most important prognostic factor is stage at time of presentation, and a melanoma is most capable of being cured by surgical excision when it is at the superficial stage (“radial” growth phase CMM, as opposed to vertical growth phase CMM; Ref. 16). Radial growth phase CMMs exist solely within the epidermis or may contain small numbers of nondividing cells within the superficial dermis. Vertical growth phase CMMs contain neoplastic melanocytes actively growing within the dermis and possess the capability to metastasize to regional lymph nodes and distant organs. In vertical growth phase CMMs, prognosis is also influenced by the depth of invasion of the lesion (Breslow and stage at presentation (I versus II/III), Breslow depth (greater than or less than 1.5 mm) and mitotic count in vertical growth phase CMM, presence of tumor infiltrating lymphocytes, and presence or absence of relapse.

**Table 1** **MMP-1 genotype and allele frequencies (%) in CMM patients, British Caucasian controls, and healthy Japanese subjects***

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Vertical growth</th>
<th>Horizontal growth</th>
<th>P</th>
<th>2G allele frequency (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2G/2G</td>
<td>41 (29%)</td>
<td>15 (34%)</td>
<td>0.0870</td>
<td>53% (47%, 59%)</td>
</tr>
<tr>
<td>1G/2G</td>
<td>65 (47%)</td>
<td>14 (17%)</td>
<td>2.23 (0.83, 5.95)</td>
<td></td>
</tr>
<tr>
<td>1G/1G</td>
<td>33 (24%)</td>
<td>2.23 (0.83, 5.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td><strong>0.4136</strong></td>
<td><strong>0.0870</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Genotype and allele frequencies in Japanese are those reported by Kanamori et al. (13).

**Table 2** **Frequency of MMP-1 genotypes in vertical and horizontal growth phase CMM**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Vertical growth</th>
<th>Horizontal growth</th>
<th>P</th>
<th>Odds ratio (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2G/2G</td>
<td>15 (34%)</td>
<td>14 (17%)</td>
<td>0.0870</td>
<td>2.23 (0.83, 5.95)</td>
</tr>
<tr>
<td>1G/2G</td>
<td>16 (36%)</td>
<td>41 (50%)</td>
<td>0.81 (0.34, 1.95)</td>
<td></td>
</tr>
<tr>
<td>1G/1G</td>
<td>13 (30%)</td>
<td>27 (33%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>2G/2G</td>
<td>15 (34%)</td>
<td>14 (17%)</td>
<td>0.0333</td>
<td>2.51 (1.08, 5.88)</td>
</tr>
<tr>
<td>1G/2G or 1G/1G</td>
<td>29 (66%)</td>
<td>68 (83%)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

* CI, confidence interval.
Orientals and Caucasians. It was noted that the cancer or a difference in genetic risk factors for cancers between patients and healthy subjects was detected. This may indicate a study, no difference in

ically predisposed to the development of ovarian cancer (13). In our

2G

subjects, suggesting that individuals carrying the

paring the

MMP-1

allele contain higher levels of MMP-1 transcripts com-

pared with those from patients not carrying this allele (13, 20). In a

researcher that ovarian tumor and endometrial cancer tissues from patients car-

rying the 2G allele have significantly higher levels of MMP-1 transcripts com-

pared with those from patients not carrying this allele (13, 20). In a

recent Japanese study of ovarian cancer, the proportion of patients

who carried the 2G allele was significantly higher than in the control

subjects, suggesting that individuals carrying the 2G allele are geneti-

cally predisposed to the development of ovarian cancer (13). In our

study, no difference in MMP-1 genotype frequency between CMM

patients and healthy subjects was detected. This may indicate a

difference in genetic risk factors between melanoma and ovarian
cancer or a difference in genetic risk factors for cancers between

Orientals and Caucasians. It was noted that the MMP-1 genotype

frequencies within our healthy Caucasian control population differed

significantly from those within the healthy Japanese subjects used in

a previous study (13).

The association of the 2G allele of the MMP-1 gene with vertical
growth phase CMM and the trend toward lower survival rate observed

in this study is consistent with the hypothesis that variation in MMP

genes can influence the potential for tumor invasion and metastasis

through modulation of the expression and/or activity of these extra-
cellular matrix-degrading enzymes. No associations were demonstra-

ble between MMP-1 genotype and the histopathological markers of

prognosis in vertical growth phase CMM that we examined. Histolog-

ical features such as Breslow depth, mitotic index, and the pres-

ence of tumor-infiltrating lymphocytes have been proven to correlate

with prognosis in vertical growth phase CMM (8). Therefore, exam-

ination of a larger patient series may be required to investigate

potential associations between MMP-1 genotype and both these more
detailed histological features and clinical outcome.

To our knowledge, this is the first report of a study of a naturally

occurring variant in an MMP gene in relation to the invasiveness of

carcinoma. The data generated in this study support the hypothesis

that variation in the MMP-1 gene influences the potential for invasion

and metastasis of CMM through modulation of the expression of this

matrix-degrading enzyme.

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Kanamori, Y., Minaguchi, T., Nakamura, Y., Tokino, T., and Kudo, R. A single


Table 3 Hazard ratios for relapse in CMM series according to MMP-1 genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Hazard ratios (95% CI*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1G/1G (n = 19)</td>
<td>1.00</td>
</tr>
<tr>
<td>1G/2G (n = 50)</td>
<td>1.12 (0.36, 3.52)</td>
</tr>
<tr>
<td>2G/2G (n = 50)</td>
<td>1.52 (0.46, 5.06)</td>
</tr>
</tbody>
</table>

* CI confidence interval.
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