Presence of matrix metalloproteinase–2 and tissue inhibitor matrix metalloproteinase–2 gene polymorphisms and immunohistochemical expressions in intracranial meningiomas

Laboratory investigation

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Object. Meningiomas are benign extraaxial tumors with a slow progression. Some of them, in spite of being benign in nature, may show an aggressive progression pattern. To investigate the behavioral characteristics of meningiomas, researchers have studied matrix metalloproteinases (MMPs), their tissue inhibitors (TIMPs), interstitial collagens, proteins, vascular endothelial growth factors (VEGF), and tumor necrosis factors.

Methods. In this study, the authors investigated MMP2 and TIMP2 gene polymorphisms in formalin-fixed paraffin-embedded tissue samples obtained from meningioma patients who had previously undergone surgery at the authors’ institution. In addition, brain invasion, Ki-67 index, and MMP-2 and TIMP-2 expressions were investigated using immunohistochemical methods. MMP2 (735C>T, 1575G>A, 1306C>T) and TIMP2 (418G>C, 303C>T) gene polymorphisms were investigated from paraffin-embedded tissue sections using the polymerase chain reaction–restriction fragment length polymorphism method.

Results. There were statistically significant differences between genotype (p = 0.001) and allele frequencies (p = 0.001 and OR 7.4 [95% CI 1.5–36.2]) in patient and control groups for MMP2 1306C>T polymorphism. The authors did not find a statistically significant difference for other polymorphisms. GA genotype was found to be more frequent when brain invasion was suspected for MMP2 1575G>A polymorphism (p = 0.006). There was not a statistically significant difference for other MMP2 or TIMP2 gene polymorphisms.

Conclusions. The authors’ results support the importance of MMPs and their tissue inhibitors in meningioma pathogenesis. In future studies, these gene polymorphisms, especially MMP2 1306C>T and 1575G>A, should be investigated for meningioma or brain invasion susceptibility in larger study groups.

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Key Words • meningioma • MMP-2 polymorphism • TIMP-2 polymorphism • immunohistochemistry • oncology

Meningiomas are basically benign extraaxial tumors. There are 2 subsets, namely atypical and anaplastic forms that account for approximately 10% of all meningiomas and show aggressive biological behavior. Meningiomas are the most common benign tumors of the intracranial cavity, constituting 13%–26% of primary brain neoplasms. The principal mode of treatment remains surgery with the aim of total tumor removal. Radiosurgery is being used with increasing frequency for certain groups of patients either as a primary or adjuvant therapy.

The difference in incidences of meningiomas between men and women has led to the idea that sex hormones and their receptors play a role in meningioma pathogenesis. The genes located in sex chromosomes could be causing meningiomas. In the literature, genetic changes such as the loss of chromosome arms 1p, 6q, 9p, 10q, 14q, and 18q and gene amplifications at 1q, 6q, 12q, 19q, and 20q have been reported.¹⁸ MADI2, MADH4, AMP-I, and DDC tumor suppressor genes located in chromosome re-

Abbreviations used in this paper: MMP = matrix metalloproteinase; PCR = polymerase chain reaction; TIMP = tissue inhibitor of MMP.
gion 18q21 have been reported to play an important role in meningioma pathogenesis. Losses in chromosome 14 are the third most frequent genetic change following chromosome 22 and chromosome 1 rearrangements. Loss of 14q has been shown to be a predictive factor in recurrences. In addition to known genetic changes in meningioma pathogenesis, HER-2/neu gene amplification has been investigated in tissue samples and concluded to be a risk factor.

The WHO has classified meningiomas 4 times: in 1979, 1997, 2000, and 2007. In the last classification (WHO 2007), criteria such as recurrence grade, potential of aggressive growth, cell type, clinical findings, and biological activity were evaluated. According to this classification, Grade I meningiomas have a lower recurrence risk with a slow growth rate. Grade II and III meningiomas have a higher recurrence risk and/or aggressive behavior.

Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) play an important role in the cancer invasion in different ways. MMPs are the family of endopeptidases, present in normal healthy tissues. The tumor microenvironment can be regulated by MMPs. MMP expression and activation are increased in almost all human cancers compared with normal tissue. Activity of MMPs is also regulated by several factors including TIMPs.

In previous studies, MMP-2 expression has been investigated in meningiomas and is associated with tumor recurrence and histological type, and might be a potential therapeutic target.

MMPs are positive regulators of angiogenesis. They enable penetration of epithelial cells to the stroma by destruction of the extracellular matrix. MMP-2, MMP-9, and MMP-14 play a direct role in angiogenesis. The factors inducing angiogenesis increase MMP production to destroy the basal membrane, which contains Type 4 and Type 5 collagen. After the invading cells cross the basal membrane, cell proliferation and invasion result in new vessel formation or new metastatic forms. Morbidity and mortality are dependent on tumor tissue in primary intracranial tumors. In benign meningiomas, collagenase activity in tumor tissue has been found to be low, whereas it is high in invaded dural and bone tissue. Cancer formation results in the disturbance of the equilibrium between cell proliferation, differentiation, and death.

In the current study, we aimed to investigate MMP2 and TIMP2 gene polymorphisms in formalin-fixed and paraffin-embedded meningioma tissues. The WHO has established the latest classification criteria for meningioma classification. Brain invasive meningiomas are defined as those that invade brain parenchyma and are either WHO grade II or III. In each case, brain invasion was noted as a separate parameter and was defined as irregular projections of the tumor into adjacent CNS parenchyma without an intervening layer of leptomeninges. According to the latest WHO classification, brain invasive meningiomas were labeled as Grade II.

Immunohistochemical Analysis

Formaldehyde-fixed and paraffin-embedded tumor tissues were sectioned. After deparaffinization and rehydration, each section was immunostained using monoclonal antibodies for Ki-67 (MIB-1 clone, rabbit polyclonal, Neomarkers), MMP-2 (Ab-3, monoclonal mouse, clone: 42–5D11, Oncogene) and TIMP-2 (Ab-2, monoclonal mouse, clone: 67–4H11, Oncogene). Immunohistochemistry procedures were carried out using the streptavidin-biotin 3-step indirect methods. For MMP-2 and TIMP-2 staining, antigen retrieval was performed in a microwave oven in 10 mM citrate buffer (pH 6.0) at 700 W for 15 minutes. Endogenous peroxidase activity was then blocked with 0.3% H2O2 for 15 minutes. After incubation for 10 minutes with 5% bovine serum albumin in Tris-buffered saline (50 mM Tris-HCl, 150 mM NaCl [pH 7.4]) for blocking of nonspecific binding, sections were incubated with primary antibodies. Sections were then incubated with peroxidase-labeled polymer for 30 minutes, followed by prepared diaminobenzidine substrate-chromogen solution. The slides were counterstained with hematoxylin and then coverslipped. Between steps, the slides were washed twice in Tris-buffered saline.

Methods

Patients

Fifty patients with a diagnosis of meningioma who had undergone surgery between 2000 and 2007 at Başkent University Ankara Hospital, in the central zone of Anatolia and Turkish ethnicity, were included in the study. Of these patients, 24, 21, and 5 harbored Grade I, II, and III meningiomas, respectively. Three-micrometer-thick paraffin sections from tissue blocks of the tumors were obtained, and immunohistochemical investigation of MMP-2 and TIMP-2 expression, brain invasion, and Ki-67 index was performed in the Department of Pathology, Başkent University. The control group comprised 100 healthy age- and sex-matched subjects from the central Anatolian region who did not have meningioma or other benign or malignant tumors or a family history of one of these tumors. Peripheral blood samples were obtained from these control subjects.

Microscopic Investigation

The tissues used in this study were 50 formaldehyde-fixed and paraffin-embedded meningioma specimens. In each case, the histopathological diagnosis of meningioma was established by standard light-microscope evaluation of sections stained with H & E. We reevaluated sections of each sample using the WHO 2007 criteria for brain tumor classification. According to this system, an atypical meningioma exhibits increased mitotic activity (4 or more mitotic figures per 10 hpf) or 3 or more of the following features: increased cellularity, small cells with a high nucleus/cytoplasm ratio, prominent nucleoli, uninterrupted patternless or sheet-like growth, and foci of “spontaneous” or “geographic necrosis.” An anaplastic meningioma exhibits obviously malignant cytology or a high mitotic index (20 or more mitotic figures per 10 hpf) in addition to the features of atypical meningioma. The 50 specimens were grouped according to tumor grade (I, II, or III). In each case, brain invasion was noted as a separate parameter and was defined as irregular projections of the tumor into adjacent CNS parenchyma without an intervening layer of leptomeninges. According to the latest WHO classification, brain invasive meningiomas were labeled as Grade II.

J Neurosurg / Volume 121 / December 2014
For negative controls, the primary antibodies were omitted and nonimmune serum was used instead. For positive controls, we stained sections of tissues that were considered suitable according to the manufacturer’s protocol.

For Ki-67 evaluation, immunoreactivity was defined as intense, diffuse, or granular nuclear staining. The level of Ki-67 immunoreactivity in each section was assessed by counting the stained nuclei in approximately 1000 cells in the regions of maximal staining using ocular microscopy. The Ki-67 index was calculated as a percentage based on the number of positive cells per total cells counted.

The extent and intensity of expression for MMP-2 and TIMP-2 were semiquantitatively evaluated. Scoring was classified into the following four groups: 0, no expression; 1, low expression; 2, moderate expression; and 3, high expression.

**Genotyping**

MMP2 (735C>T, 1575G>A, 1306C>T) and TIMP2 (418G>C, 303C>T) gene polymorphisms were investigated from paraffin-embedded tissue sections using the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. DNA was extracted from tissue and blood samples using commercially available kits. Concentrations of genomic DNA samples obtained from tissue sections of patients and peripheral blood samples of the control group were spectrophotometrically measured, and approximately 250 ng of DNA was used for each reaction. The PCR products were electrophoresed on 2% agarose gels at 90 V for 50 minutes. PCR products were quantified for restriction enzyme reactions depending on the band brightness (5–20 μl), and, accordingly, PCR products were incubated with 5 U of restriction enzymes. Then, products were checked by electrophoresing on 2% high-resolution agarose gels at 90 V for 2 hours.

**Statistical Analysis**

The SPSS (Statistical Package for the Social Sciences) package program was used for statistical analysis of data. Descriptive statistics were expressed as mean ± standard deviation for continuous data, and the number of observations and percentage for categorical data. Odds ratios, 95% confidence intervals, and significance levels were calculated using the chi-square test for determining allele frequencies; p < 0.05 was considered statistically significant.

**Results**

There were statistically significant differences between genotype (p = 0.001) and allele frequencies (p = 0.001 and OR 7.4 [1.5–36.2]) of patient and control groups for MMP2 1306C>T polymorphism. We did not find statistically significant differences for the other polymorphisms.

GA genotype was found to be more frequent when brain invasion was suspected for MMP2 1575G>A polymorphism (p = 0.006). There was not a statistically significant difference for other MMP2 or TIMP2 gene polymorphisms.

According to ANOVA analysis, the mean Ki-67 index was statistically different between types of meningiomas.

**Discussion**

Although the vast majority of intracranial meningiomas are benign and amenable to surgery, there is still a subset of tumors designated as Grade II and Grade III by WHO classification that behave in an aggressive manner with frequent recurrences and an unfavorable prognosis. Intensive research in different fields of medicine and related sciences is helping researchers to gain a better understanding of meningioma pathogenesis and is also helping them to develop reliable predictors of meningioma behavior, which may result in more effective methods of treatment. Contents and structure of intra- and extracellular matrix components and protein structure and functions have been examined by electron microscopy, molecular biology, and immunohistochemistry methods.

MMPs are proteolytic enzymes that can destroy the basal membrane and connective tissues. These enzymes are important for tissue breakdown in the process of invasive growth and metastasis.24 The inhibition of MMPs can be an alternative treatment for preventing brain invasion. Angiostatin, which is a product of plasminogen, has been shown to inhibit endothelial cell proliferation and metastatic tumor cell growth. It has also been shown that human MMPs generate biologically functional angiostatin from plasminogen. MMP-12 was the most efficient angiostatin-producing MMP.3 Downregulation of MMP activity has been demonstrated to have a striking effect on local invasion and partial suppression of hematogenous metastasis.3 Miyake et al. have studied the balance between MMP-2 and TIMP-2 in the progression of renal cell carcinoma.10 Their results suggested that the MMP-2/TIMP-2 expression ratio is an important factor. In the majority of schwannomas, meningiomas, and pilocytic meningiomas, TIMP-2 expression has been demonstrated. TIMP expression revealed a correlation with tumor grade.5,12

Nordqvist et al. analyzed the expression of MMP-2 and MMP-9 in meningiomas associated with different degrees of brain invasion and edema.14 They found that the expression of MMP-9 mRNA was identified in 14 of 16 tumors and thus a distinct correlation with increasing tumor invasion into the brain was observed while no such correlation was found with MMP-2. Okada et al. found that MMP-2 and MMP-9 expressions are prognostic factors predicting meningioma recurrence regardless of proliferative potential.9

There are studies about the possible relationship between the Ki-67 index and meningioma recurrence, metastasis, and invasion. The Ki-67 index was found to be higher in recurrent meningiomas than in nonrecurrent ones.25 In contrast to this finding, Maes et al. found Ki-67 to be a good marker of the cell proliferation status of the tumors but found no correlation with recurrence.9
MMP2 and TIMP2 gene polymorphisms

They observed that human telomerase catalytic subunit (hTERT) alone seemed to be a potential predictor of recurrence. In our study, the statistical results showed that the Ki-67 labeling index was highly correlated with meningioma grading but not with brain invasion.

To our knowledge, this is the first time MMP2 and TIMP2 gene polymorphism in meningioma patients has been studied. These polymorphisms have been studied before in several cancer types.16,20 We showed a statistically significant difference between the patient and control groups’ genotype disturbance and allele frequencies for MMP2 1306C>T polymorphism in the entire study cohort. In a meta-analysis, MMP2 1306 TT and CT genotype carriers were less susceptible to lung, head and neck, and gastric and esophageal cancer.16 In contrast, our results suggested that CT genotype and T allele frequencies associated with susceptibility for this tumor.8,20 These adverse findings in previous and present studies can be explained either by different ethnic variations in the study groups or distinct pathophysiological patterns of different cancer types.15

As mentioned before, the inhibition of MMPs can be a treatment alternative for preventing brain invasion. We suspected a statistically significant difference in patients for MMP2 1575G>A polymorphism with or without brain invasion.

Another finding was an association between all polymorphisms of MMP-2 and TIMP-2, and enhanced immunostaining. It may be concluded that better understanding of polymorphisms of MMP2 and TIMP2 may be beneficial for understanding the genetic mechanisms of meningioma pathogenesis.

Conclusions

Our results support the importance of MMPs and their tissue inhibitors in meningioma pathogenesis. In future studies, these gene polymorphisms, especially MMP2 1306C>T and 1575G>A, should be investigated for meningioma or brain invasion susceptibility in larger study groups.

Disclosure

This study was supported by the Başkent University Research Fund. The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Coven, Şahin, Alinars. Analysis and interpretation of data: Coven, Ozer, Ozen, Şahin, Alinars. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Coven. Statistical analysis: Ozer, Şahin. Administrative/technical/material support: Ozen, Alinars. Study supervision: Coven, Ozen, Şahin, Alinars.

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