Matrix metalloproteinase-2 gene polymorphism is not associated with increased glioblastoma multiforme susceptibility: An Indian institutional experience

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Abstract

Background: Glioblastoma multiforme (GBM) is a highly malignant central nervous system tumor that is extremely refractory to therapy due to its rapid growth and local invasive potential. The ability of glioma cells to invade the surrounding tissue has been attributed to the expression of matrix metalloproteinase-2 (MMP-2) in human gliomas. The -1306C/T polymorphism in the MMP-2 gene has been found to be associated with gastric adenocarcinoma, lung cancer and various other cancers including GBM. Racial and ethnic variations are known in such genetic polymorphisms.

Aims: This prospective, case control study was aimed to find out an association of MMP-2 gene polymorphism with susceptibility to develop glioblastoma in Indian population.

Material and Methods: MMP-2 gene polymorphism was studied using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 110 GBM patients and 150 healthy controls. The SPSS 17.0 statistical software (Chicago, IL, USA) was used for data management and analysis.

Results and Conclusions: A significant association of MMP-2 (−1306C/T) polymorphism with GBM (P = 0.475) was not found, suggesting that MMP-2 (−1306C/T) polymorphism is not associated with increased GBM susceptibility.

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Introduction

Glioblastoma, the most common intrinsic malignant brain tumor, [1] is characterized by excessive proliferation, high invasion of the surrounding brain tissue, and suppression of anti-tumor immune surveillance, which contribute to the malignant phenotype of gliomas. Even low-grade gliomas infiltrate the contiguous brain; a key feature which precludes their successful therapy. Interaction of integrins expressed on glioma cells with the extracellular matrix and the activity of metalloproteinases (MMPs) are prerequisites for the migration and invasion of glioma cells. [2],[3] MMPs are a growing family of zinc-dependent endopeptidases belonging to
subfamily M10A, which are capable of degrading various components of the extracellular matrix including collagens, laminin, fibronectin, vitronectin and proteoglycans. These enzymes have been implicated in a variety of physiological and pathological conditions including embryogenesis, tumor invasion and metastasis. The regulation of MMP expression is complex. The synthesis of many MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), is thought to be regulated by growth factors, cytokines and hormones. MMP-2 and MMP-9 have been well studied in gliomas, particularly because these enzymes are easy to measure by gelatin zymography. A large body of literature reports that MMP-2 and MMP-9 are upregulated in glioma specimens in vitro and in vivo.[4],[5],[6],[7],[8] MMP-2, a 72 kDa gelatinase, degrades non-fibrillar and denatured collagens (primarily type IV collagen), and is secreted as a latent pro-form which is processed into the active molecule through interaction with membrane-type1-MMP (MT1-MMP) on the cell surface, and the interaction is regulated by TIMP. [9]

MMP-2 also has high activity toward many other bioactive molecules such as growth factor-binding proteins and growth factor receptors, which are well known to have a strong effect on stimulating cell proliferation and inhibiting apoptosis. These activities of MMP-2 are believed to be linked to both cancer development and progression. [10] The cancers in which an effect for MMP-2 has been established are characterized by varying individual susceptibility, implying the role of genetic factors. However, like many MMPs, MMP-2 is not upregulated by gene amplification or activating mutations, and genetic alternations in the gene of the cancer cells are generally lacking. Therefore, germ-line polymorphisms that alter constitutive and/or induced expression and enzyme activity of MMP-2 may affect individual susceptibility to certain cancers. [11]

Price et al., described the nature and extent of nucleotide variation in the human MMP-2 gene by identifying 15 novel single nucleotide polymorphisms (SNPs) distributed throughout the coding and non-coding regions. The common C → T transition at −1306, which disrupts an Sp1-type promoter site (CCACC box), displayed a strikingly lower promoter activity with the T allele. [12] This suggests that this common functional genetic variant influences MMP-2 gene transcription in an allele-specific manner and is therefore an important candidate to test for association in a wide spectrum of pathologies for which a role for MMP-2 is implicated, including atherogenesis and tumor invasion and metastasis. In fact, it has been shown in various studies that frequency of −1306 MMP-2 (C/T) polymorphism is significantly higher in patients of lung cancer, gastroesophageal cancer, breast cancer, invasive bladder cancer and cervix cancer. [13],[14],[15],[16],[17],[18],[19]

The data on strength of association between −1306 MMP-2 polymorphism and glioblastoma multiforme (GBM) in Indian population are not available. Therefore, the present study was carried out to look for the association of MMP-2 gene polymorphism with susceptibility of developing glioblastoma in Indian population. The study, in addition, also provides −1306 MMP-2 genotype pools in normal Indian population as a baseline for further studies on the subject pertaining to various diseases.

Material and Methods

Study population

This study subjects included 110 patients with GBM (mean age ± SD, 37.01 ± 14.62 years; male:female, 81:29) and 150 age-gender matched healthy controls (mean age ± SD, 39.87 ± 11.85). All subjects were ethnic Indians. GBM patients were selected on the basis of histopathological confirmation of the tumor tissue by the department of pathology. In the patient population, we did not include any patient who was reoperated or with an evidence of malignant transformation from a lower grade to GBM or with a presenting history of longer duration (>4 months). The Institutional Ethics Committee (IEC, SGPGIMS) granted approval for the study and informed consent was obtained from all the study subjects.

DNA extraction

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood by salting-out method [20] and stored at −20°C till further use. DNA samples of 10 ng/µl concentration were used
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for the detection of SNPs.

**MMP-2 (−1306 C/T) genotyping**

MMP-2 genotypes were determined by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based method, as previously described. [21] The primer sequences for MMP-2 were: forward 5′-ATAGGGTAACCTCCCACATT-3′ and reverse 5′-GGTAAAATGAGGCTGAGACCTG-3′ (Bangalore Genei, India). All PCR amplifications were performed in a 20 µl volume containing 10× assay buffer, 200 µM each dATP, dCTP, dGTP, dTTP, 0.1 µM each primer, and 1.0 U Taq DNA polymerase (Bangalore Genei, India). PCR conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 30 seconds, followed by final extension step at 72°C for 5 min and cooling to 4°C. Template free water was used as a negative control. After amplification, the purified 4 µl PCR products were subjected to restriction digestion by Eco RI restriction endonuclease (Fermentas, Vilnius, Lithuania) for MMP-2 polymorphism with 1 µl 10× enzyme buffer (Fermentas) overnight at 37°C. The digested DNA fragments were then separated on 3% agarose gel electrophoresis. After digestion, fragment sizes for carriers of the polymorphic allele decreased from 300 bp (wild-type) to 300 bp, 254 bp, and 46 bp (heterozygous) and to 254 bp, 46 bp (variant). All the experiments were repeated twice for confirmation of the RFLP results. No discrepancy was found after sequencing of the 10% randomly selected samples.

**Statistical analysis**

The SPSS 17.0 statistical software (Chicago, IL, USA) was used for data management and analysis. Hardy-Weinberg equilibrium was checked in controls by goodness of fit c2 test. For comparisons between the groups of study populations, χ2 test was used. Logistic regression analysis was applied to estimate association with GBM susceptibility after adjusting for age and gender. P value ≤0.05 was considered significant.

**Results**

**Characteristics of GBM patients and control subjects**

In present study, age was comparable in GBM patients and control groups (39.87 ± 11.85 vs. 37.01 ± 14.62 years, P > 0.05). No significant difference was present with respect to gender (male/female = 81/29 in patients and 99/51 in controls (P > 0.05 for both) [Table 1]. The MMP-2 polymorphism was in agreement with Hardy-Weinberg equilibrium in control subjects. [Table 1]

**MMP-2 (−1306C/T) polymorphisms and GBM**

The genotype frequency was compared between patients and controls to analyze the association of MMP-2 (−1306C/T) polymorphism with GBM susceptibility. The genotypic distribution of MMP-2 (−1306C/T) polymorphism demonstrated no association of GBM susceptibility in patients with C/T (OR, 0.807; P = 0.475; 95% CI, 0.44-1.45) as well as with T/T (OR, 1.19; P = 0.865; 95% CI, 0.16-8.94) genotypes compared to normal subjects. Patients with GBM showed almost similar prevalence of T allele of −1306 MMP-2 gene polymorphism (12.7 vs. 14.3%, OR, 8.73; P = 0.598; 95% CI, 0.52-1.45) compared to controls [Table 2]. [Table 2]

**Discussion**

The present study was aimed to find out the association of MMP-2 (−1306C/T) gene polymorphism with GBM susceptibility. We showed for the first time that −1306 MMP-2 gene polymorphism is not significantly associated with GBM patients compared to controls from northern Indian population. However, an association of −1306 MMP-2 gene polymorphism with various other malignancies has been demonstrated. [13],[14],[15],[16],
MMP-2 (gelatinase A) has type IV collagenolytic activity and is constitutively expressed by most connective tissue cells including endothelial cells, osteoblasts, fibroblasts and myoblasts. The membrane-bound activation of pro-MMP-2 through its proteolytic activity against components of the basement membrane executes extracellular matrix remodeling as well as uniquely generates several different biologically active molecules including laminin, fibronectin, and monocyte chemoattractant protein-3. Majority of studies concerning MMP-2 have focused on demonstrating its essential role in promoting cell invasiveness during tumor angiogenesis as well as tumor metastasis.

GBM is a highly malignant central nervous system tumor that is extremely refractory to therapy due its rapid growth and local invasive potential. This rapid infiltrative growth prevents successful surgical resection of GBM. The ability of glioma cells to invade the surrounding tissue has been attributed to their secretion of MMPs. In vivo studies have demonstrated the expression of MMP-2 in human gliomas. MMP-2 expression was the highest in high grade gliomas (GBM, anaplastic astrocytoma) as compared to low grade astrocytomas and normal brain.

Cancers in which a role for MMP-2 has been demonstrated are generally characterized by various individual susceptibilities, implying the role of genetic factors. A functional SNP in MMP-2 has been reported: a −1306C→T transition in the MMP-2 promoter sequence disrupts an Sp1-type promoter site (CCACC box), and thus displays a strikingly lower promoter activity with the T allele in an in vitro assay system. It has been suggested that the −1306C/T polymorphism in the MMP-2 gene may be informative in a test of association with cancer development in which a role for MMP-2 is implicated. This functional polymorphism has been demonstrated in various cancers. If this polymorphism actually causes variations in transcription and expression of MMP-2 in vivo, it might affect individual susceptibility to carcinogenesis.

The variant allele (T) of this polymorphism was not significantly associated with susceptibility of developing GBM in our study population. Such genetic polymorphisms are known to be affected by racial and ethnic variations. To add, this study also shows that CC is the most frequent genotype of −1306 MMP-2 gene polymorphism in both GBM as well as in normal subjects (76.4 and 72.7%, respectively). These data on genotype pool shall be useful for further studies on Indian population.

Although the −1306 MMP-2 gene polymorphism has been shown to be associated with other cancers such as esophageal, lung, breast, prostate, invasive bladder cancer and gastric cardia adenocarcinoma as well as brain cancer, however, this pilot study has not shown any association of −1306 MMP-2 gene polymorphism with GBM in Indian population (P = 0.475). This disagreement in the present study is most likely due to small sample size and also can be attributed to racial and ethnic variations in different populations. It would therefore be rational to confirm these findings by a large population-based prospective study to settle the controversy. The results, however, may also be a pointer toward the greater significance of haplotypes of MMP-2 gene with genes on adjacent loci rather than SNP of MMP-2 gene only. In addition, other factors such as exposure to toxic chemicals, radiation, and electromagnetic fields have been implicated by some in the pathogenesis of these tumors. These factors might interact with MMP-2 genotype or act as potential confounders in the analysis. Unfortunately, information on these factors in this study was not available.

To summarize, this study remains a pilot study to find the association of MMP-2 gene polymorphism with GBM and more efforts in different geographic ethnic groups are required in larger population to reach a firm conclusion.

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