An Overview of Matrix Metalloproteinase 9 Polymorphism and Gastric Cancer Risk

Sugreev Verma¹, Kousik Kesh¹, Arnab Gupta², Snehasikta Swarnakar¹*

Abstract

Matrix metalloproteinase (MMP) 9, a key member of multifunctional family of zinc dependent endopeptidases has been found to be upregulated during inflammation and in some cancers. MMPs cleave extracellular matrix (ECM) proteins and play critical roles in cellular apoptosis, angiogenesis, tumor growth and metastasis. Several genetic polymorphisms have been identified that show allele specific effects on MMP9 regulation and are associated with gastric cancer, the fourth most common malignancy in the world. Besides Helicobacter pylori infection, genetic predisposition is another documented risk factor for gastric carcinoma. The single nucleotide polymorphism (SNP) at position -1562C/T of MMP9 results in the modulation for binding of transcription factors to the MMP9 gene promoter and thereby causes differences in protein expression and enzymatic activity. MMP9 transcriptional regulation during gastric cancer development remains poorly known although several studies have demonstrated associations between MMP9 -1562 C/T polymorphism with different diseases. Knowledge on mechanisms of MMP9 upregulation during gastric cancer may provide new paradigm in diagnostics and therapeutics.

Keywords: Gastric cancer - matrix metalloproteinase 9 - single nucleotide polymorphism - case control study.

Introduction

During 1980s the most frequent type of cancer was the cancer of stomach (Wu et al., 2002). Until recently stomach cancer is the second most cancer worldwide with approximately 870,000 new cases per year (Sentani et al., 2008; Ferlay et al., 2010). Gastric cancer incidence rates vary up to ten fold throughout the world. Nearly two third of gastric cancers occur in developing countries (Crew and Neugut, 2006). However, gastric cancer remains a prevalent cancer in eastern part of the world; Taiwan. Gastric cancer ranks as the fourth highest cause of cancer related death with a mortality rate of 10.72 per 100000 (Wu et al., 2002). In India, nearly one million new cancer cases were estimated in recent years (Murthy et al., 2008).

Although the incidence of gastric cancer is declined in western countries but it remains fourth most common cancer worldwide. The decrease in the incident of gastric cancer is associated with standard of living, proper dietary habits and adequate intake of vitamins. Over the past years, a great deal of research has clarified the details of genetic and epigenetic abnormalities related to gastric cancer development (Choi and Wu, 2005). Infection with H. pylori is the major cause for pathogenesis of gastric cancer. Smoking and tobacco intake doubles the risk for gastric cancer development. Moreover, genetic predisposition, diet, stresses and environmental factor accounts for other risk factors.

MMPs are essential regulators of the microenvironment of the cell through there capability of degrading ECM which is considered as a barrier in cellular invasion (Sternlicht and Werb, 2001). MMPs are synthesized as inactive zymogens (pro-MMPs) by several types of cells and become activated in the extracellular space by other MMPs or serine proteases including plasmin (Inuzuka et al., 2000). The ECM of the gastric mucosa is composed of a number of macromolecules, such as collagen, laminin, proteoglycan, elastin, fibronectin and hyaluronic acid, and their degradation by MMPs plays an important role in maintaining the cellular microenvironment (Hellmig et al., 2006). MMP9 (92- kDa, type IV collagenase) located on human chromosome 20q11.1-13.1 (Fig1), is a key enzyme in the causation of gastric ulcer (Swarnakar et al., 2005; Kundu et al., 2006). MMP9 (also known as gelatinase B) can degrade collagen type IV, collagen type V and elastin as well. MMP9 is mainly expressed by alveolar macrophages, polymorphonuclear leukocytes, osteoclasts and malignant cells (Fanjul-Fernández et al., 2010). Under pathological conditions including gastro-intestinal inflammation and gastric cancer, enhanced level of MMP9 has been described. Increased expression of MMP9 mRNA has been documented in primary tumor and metastatic tissues of gastric cancer (Sier et al., 1996). H. pylori infection of gastric carcinoma cells showed increased...
mRNA and protein level of MMP9 (Fox and Wang, 2007). Increased MMP9 expression and transcription that might be the result of diminished repressor binding to the promoter region. A comprehensive literature survey revealed that MMP9 SNP was significantly associated with various diseases being cardiovascular diseases rank first and stomach diseases rank sixth (Fig2).

Several epidemiologic studies revealed the association of MMP9 and development as well as progression of gastric cancers in different populations (Sier et al. 1996; Zhang et al., 2012). Over expression of MMP9 has been observed in a variety of cancer including gastric cancer and its expression is associated with pathological features, such as TNM stage, lymphatic invasion, tumor depth (Zhang et al., 2003). Tan et al reported that the serum MMP9 level increased gradually along with the depth of tissue invasion in gastric cancer patients (Tan et al., 2007).

Various Polymorphisms of MMP9

Genetic polymorphism describes existence of two or more different genotype or allelic variant in a population. SNP is the most common DNA sequence variation, which accounts for 1 in 100 in the genetic polymorphism. Genetic polymorphisms of MMPs are being increasingly recognized in the context of etiology and pathogenesis of gastric cancer. In human genome, the estimated number of SNP is 10 million while a small part is functionally relevant. Functional SNPs are mainly located in the promoter region. However, most of the studies showed that polymorphisms in MMP9 promoter region are more relevant to disease progression (Langers et al., 2011). In normal physiological conditions, genes are tightly regulated by transcription factors, whereas in cancer, aberrant activities of transcription factors deregulate the gene expression, leading to metastasis (Libermann and Zerbini, 2006). Therefore, the comprehensive knowledge of transcription factor binding sites (TFBS) in promoter region is essential for inferring gene regulatory networks (Hannenhalli, 2008). The manner and extent to which genetic factors contribute to disease have important implications for identifying the genetic basis of etiology and for utilizing this information for the diagnosis and therapies. Complex human diseases like cancers show relatively mild phenotype and are slowly progressive and chronic in nature.

Furthermore, the pathology is usually not clinically evident until in advanced stages. Complex diseases are typically polygenic and might have multiple gene associations, which individually have weak effects but when combined with each other and external influences, such as environmental factors, result in variable disease manifestation. The reasons behind an association of a disease phenotype with a haplotype instead of individual polymorphism are i) a functional effect on gene expression is dependent on interaction between two or more polymorphism ii) generally haplotype has a higher probability than individual polymorphisms of showing useful linkage disequilibrium with an unknown causal variant.

To date, most studies focus on “functional” SNPs, but the number of SNPs with clear function is limited and incorporating SNPs in studies of cancer predisposition and prognosis to find out the true association are still challenging tasks. Regulatory SNPs, which are in the promoter region specifically, alter the binding affinity of transcription factor to DNA and in turn contributing to disease phenotype.

MMP9 gene promoter contig sequence confirmed the polymorphism positions -108 [(CA)n repeats] -1562C/T, -1831T/A and -1932C/T. A cytosine (C) to thymine (T) transition at nucleotide -1562 in the promoter of MMP9 gene generates low activity for C/C and high activity for C/T and T/T genotypes in gene transcription. In addition, there are polymorphic positions in the coding region of MMP9 as reported in different studies, for example R279Q, P574R, R668Q sites are documented in MMP9 structural region (Hu et al., 2005; Tang et al., 2008).

The distribution of genetic variants of MMP9 in Indian population was found to be different than that in Japanese, Korean, Chinese, Caucasian and African-American populations (Hirakawa et al., 2003; Kubben et al., 2006; Woo et al., 2007; Xing et al., 2007; Lee et al., 2009). Recently, haplotype-based association study has been proposed as a powerful and comprehensive approach to identify causal genetic variation underlying complex diseases. Figure 1 shows pictorial depiction of SNPs in MMP9 gene covering 13 different SNPs in 5' UTR, 29 SNPs in structural gene and 11 SNPs in 3' UTR.

Gastric Cancer Risk and MMP9 Polymorphisms

MMPs alter the tumor microenvironment and may affect the process of carcinogenesis and, they appear to be induced through transcriptional activation (Ye et al., 1996). Being a member of MMP family, MMP9 has been reported to play an important role in cancer invasion through their over expression, which is associate with metastasis and unfavorable prognosis in gastric cancer (Inoue et al., 1999; Kanamori et al., 1999; Ghilardi et al., 2001). Degradation of basement membrane is one of the major characteristics of gastric cancer, and is mediated by different MMPs, including MMP9. Invasion of surrounding structure and lymphatic metastasis are the main factors influencing the prognosis and survival of gastric cancer patients (Zhang et al., 2004a). Various studies revealed the association of MMP9 variants and risk of gastric cancer development.
and progression (Langers et al., 2011).

Recent studies have demonstrated that degradation of ECM and basement membrane by MMPs play an important role in tumorigenesis by modulating cell proliferation, apoptosis, and host immune surveillance, tumor invasion and metastasis (Kohn and Liotta, 1995; Forget et al., 1999). The polymorphisms of MMPs either in separate or combination are closely correlated with gastric cancer risk with age, sex and addiction. MMP9 -1562 C/T genotyping was performed by PCR-RFLP analysis using Sph1 restriction endonuclease (Figure 3). Subjects with MMP9 -1562 CT or TT genotype were at higher risk of gastric cancer as compared to MMP9 -1562 CC genotype in eastern Indian populations (Table 1). There is direct evidence of higher promoter activity of CT or TT allele as compared CC allele, thus allowing more transcription of MMP9 gene for CT/CT individuals. Table 1 shows the association of MMP9 -1562 C/T polymorphism with gastric cancer risk. A total of 463 samples from patients with gastric cancer and control were examined in the study for the genotyping of MMP9 promoter. Patients with higher age group (> 50 years), with CT genotype displayed a significant difference in distribution than controls and associated with significant risk for gastric cancer development (p=0.001, OR=1.841, CI=1.267-2.68).

The -1562 C/T SNP located at the promoter of MMP9 is considered as potential genetic factor for progression of gastric cancer because it directly affects the transcription of MMP9 gene (Matsumura et al., 2005).

The MMP9 gene promoter contains binding sites for AP-1, NF-kB, Sp-1 and Ets-1 transcription factors (Gum

![Figure 2. MMP9 SNPs and Major Diseases: A Survey of HUGE Navigator.](image)

![Figure 3. Genotyping of MMP9 -1562 C/T Polymorphism.](image)

**Table 1. Analysis of Association between MMP9 -1562C/T Polymorphism and Gastric Cancer Risk in Eastern Indian Populations**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n Patient</th>
<th>%</th>
<th>n Controls</th>
<th>%</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>230</td>
<td>46.9</td>
<td>143</td>
<td>35.1</td>
<td>3.51</td>
<td>(1.841)</td>
<td>0.001</td>
</tr>
<tr>
<td>CT</td>
<td>114</td>
<td>49.5</td>
<td>82</td>
<td>3.4</td>
<td>3.4</td>
<td>(1.324)</td>
<td>0.611</td>
</tr>
<tr>
<td>TT</td>
<td>8</td>
<td>3.4</td>
<td>8</td>
<td>3.4</td>
<td>3.4</td>
<td>(1.324)</td>
<td>0.611</td>
</tr>
<tr>
<td>CT+TT</td>
<td>122</td>
<td>53.04</td>
<td>90</td>
<td>23.7</td>
<td>23.7</td>
<td>(1.795)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Odd ratio (OR) was calculated by binary logistic model using Graph pad In stat software to measure the strength of association of disease occurrence. p value was calculated by chi square to know significance in the distribution of genotype between patient and control. Significant values are shown in bold. CI=confidence ratio, Ref=Reference genotype for calculation of OR.
Table 2. Association of MMP9 -1562 C/T Polymorphism and Risk of Gastric Cancer in Various Ethnic Population

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Journal, Country</th>
<th>SNP position</th>
<th>Genotype assay</th>
<th>Subjects (Case/Control)</th>
<th>Study parameter</th>
<th>Principle finding</th>
<th>OR (Odd Ratio)</th>
<th>95% CI (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang XM 2004</td>
<td>Ai Zheng, China</td>
<td>−1562C/T</td>
<td>PCR-RFLP</td>
<td>228/774</td>
<td>Cancer risk</td>
<td>No association with cancer risk</td>
<td>CT+TT vs CC 2.61</td>
<td>1.07-6.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCR-HPLC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CT+TT vs CC =2.27</td>
<td>1.09-4.74</td>
</tr>
<tr>
<td>Matsumura 2005</td>
<td>Japan</td>
<td>PCR-RFLP</td>
<td>Sequencing</td>
<td></td>
<td>Cancer risk, T allele associated with tumor invasion, lymphatic invasion and clinical stage.</td>
<td>No link with cancer risk</td>
<td>CT+TT vs CC 2.66</td>
<td>1.12-4.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PCR-RFLP</td>
<td>R279Q</td>
<td>74/100</td>
<td>Cancer risk, Lymph node metastasis</td>
<td>No association with cancer risk</td>
<td>RR vs QQ+RQ 5.74</td>
<td>-0.02</td>
</tr>
<tr>
<td>Tang Y 2013</td>
<td>Clin Cancer Res</td>
<td>China</td>
<td>R279Q</td>
<td>74/100</td>
<td>Cancer risk, Lymph node metastasis</td>
<td>No association with cancer risk</td>
<td>PP vs RR+PR 4.17</td>
<td>1.59-13.43</td>
</tr>
<tr>
<td>Krishnaveni D 2012</td>
<td>India</td>
<td>−1562C/T</td>
<td>Tetra-primer amplification refractory mutation PCR</td>
<td>140/132</td>
<td>Cancer risk, Epidemiology of risk factor</td>
<td>Increased T allele in cancer, smoking enhanced cancer risk in TT than CC genotype</td>
<td>1.39-11.78</td>
<td></td>
</tr>
<tr>
<td>Lee TY 2013</td>
<td>Clin Cancer Res</td>
<td>China</td>
<td>P574R</td>
<td>74/100</td>
<td>Cancer risk, Lymph node metastasis</td>
<td>No association with cancer risk</td>
<td>CT+TT vs CC 2.12</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.47(HR) 2.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zhang Wei-qiang</td>
<td>China</td>
<td>−1562C/T</td>
<td>PCR-RFLP</td>
<td>170/200</td>
<td>Cancer risk, Lymphatic metastasis, Tumor staging</td>
<td>No link with cancer risk</td>
<td>CT+TT &gt;CT+TT in higher stage</td>
<td>0.02</td>
</tr>
<tr>
<td>KH Hung 2009</td>
<td>Helicobacter</td>
<td>Tiwan</td>
<td>PCR-RFLP</td>
<td>296/0 (Hp-infected)</td>
<td>Risk of Intestinal metaplasia</td>
<td>Combination of MMP9 −1562C/T and TIMP1−372 CC/T+CT/T in intestinal metaplasia</td>
<td>1.59-13.43</td>
<td></td>
</tr>
</tbody>
</table>

A comprehensive literature search was done using electronic data bases of Pubmed, ISI web of knowledge, Medline and google scholar in terms of MMP9 -1562 C/T polymorphism and risk of gastric cancer, serosae invasion, lymphnode metastasis, survival, tumor staging

are different between Asian and Caucasian populations (Joos et al., 2002).

MMP9 Polymorphism in Various Populations

The gastric cancer rates show marked geographical variation, with high-risk areas in Japan, China, eastern Europe and certain countries in Latin America. Low-risk population is seen among whites in North America, India, Philippines, most countries in Africa, some western European countries and Australia. In India, the number of new stomach cancer cases in 2001 was estimated to be approximately 35,675 (n=23,785 in men; 11,890 in women) (Dikshit et al., 2011). These differences in incidence rates can be attributed to many factors but refer particularly to differences in dietary habits, infection by Helicobacter Pylori and presence of intra individual genetic predisposition factors. Like most cancers, gastric cancer has a complex multistep etiology that involves both environmental and genetic factors. MMP9 is frequently overexpressed in gastric cancer and the gene expression mainly ntrrolled at transcriptional level. Hence, the presence of SNP within promoter region markedly influences gene expression as well as disease susceptibility. As gastric cancer showed marked geographical variation and mostly prominent among Asian population, most of the studies concerning MMP9 polymorphism and gastric cancer risk were conducted in Asian population.
Zhang et al investigated the association of functional polymorphisms in the MMP2 and MMP9 genes with risk of gastric cancer in a Chinese population. The effect-modified model was used to evaluate the gene-gene interaction. They did not find significant association between MMP9 -1562C/T polymorphism and risk of gastric cancer. However, the polymorphisms in these two genes seem to display a gene-gene interaction, with a high cancer risk for subjects carrying both MMP-2 -1306CC and MMP9 -1562TT or CT genotypes compared with those carrying both MMP-2 -1306TT or CT and MMP9-1562 CC genotypes (Zhang et al., 2004b).

Matsumura et al reported -1562C/T SNP in the MMP9 promoter affecting tumor progression and invasive phenotype of gastric cancer among Japanese population (Matsumura et al., 2005). They found that genotype frequencies in gastric cancer patients were similar to those in control subjects, however the presence of T allele at MMP9 -1562 site was significantly associated with increase tumor invasion, more advance stage of cancer, lymphatic invasion and deeper submucosal infiltration in gastric cancer patients. On the other hand, Kubben et al did not find any positive association between MMP9 -1562 C/T polymorphism and gastric cancer risk among Caucasian population (Kubben et al., 2006). Association of two non-synonymous SNP (R279Q and P574R) located at the exon region of MMP9 gene on the occurrence and progression of gastric cancer has been studied by Tang et al in Chinese population (Tang et al., 2008). These two coding region polymorphisms are associated with amino acid substitution in the MMP9 protein. R279Q SNP substitute an Arg to Gln in fibronectin type II domain and P574R SNP is associated with Pro to Arg substitution within the hemopexin domain. A significant association between the above two non-synonymous MMP9 polymorphisms with lymph node metastasis in gastric cancer, especially with the diffuse type was observed suggesting specific role of MMP9 protein in lymph node metastases. Kim et al reported that the allele and genotype frequencies of MMP9 rs17576 (Table 2) were not associated with the development of gastric cancer and lymph node metastasis in Korean population (Kim et al., 2011). Significant correlation with MMP9 -1562 C/T or T/T genotype and higher risk of gastric cancer among female in Taiwan was documented by Lee et al (Lee et al., 2013). Stratified analysis showed only elderly females with T allele had higher risk of gastric cancer. Lee et al concluded that MMP9 -1562 promoter polymorphism with T allele may be used as a marker to predict gastric cancer development in female subjects, especially in the elderly. In Indian scenario, Krishnaveni et al showed an increased frequency of T allele in the diseased compared to control subjects among south Indian population (Venkateshwari et al., 2011). We conducted a hospital based case control study to evaluate the association of MMP9 promoter polymorphism with gastric cancer risk in east Indian population. We found that MMP9 -1562 C/T polymorphism is significantly associated with gastric cancer risk (OR 1.324, 95% CI 1.24-2.59) in east Indian case-control cohort (n=463) (Table 1).

Immunohistochemical and in situ hybridization studies as well as quantitative assays have demonstrated that gastric carcinomas contain enhanced amounts of MMPs (Nomura et al., 1995; Honda et al., 1996). Gain of 20q12-1q13, where MMP9 is located, has been reported to be one of the most frequent regions with genomic gains in gastric lymph node metastasis of gastric cancer (Tang et al., 2008).

Expression of MMP9 is regulated primarily at the level of transcription, where the promoter of the gene responds to different regulators such as interleukin-1, platelet-derived growth factor, tumor necrosis factor-α, and epidermal growth factor (Zhang et al., 1999). Recent advances in genomic science have enabled us to uncover the molecular mechanism of stomach carcinogenesis and its progression. Allele specific effect of MMP9 -1562 C/T site may also influence the timing and choice of chemotherapy for patients suffering from gastric cancer. Analysis of genetic polymorphisms will give information on cancer risk and sensitivity to chemotherapy, and predict the biological behavior of the cancer. Further study of the biological ramifications of these polymorphisms may add to our understanding of the biology of gastric cancer and provide potential foci for targeted therapies and may one day be used as a risk assessment tool. The data of MMP9 SNP and gastric cancer might have utility in future development of approaches for identifying at-risk individuals and strategies to optimize treatment. A combination of examinations of different SNPs in different gene can not only foretell the patient’s prognosis but can also clarify the characteristics of the individual tumor and...
The authors acknowledge Dr. Sanjib Dey for providing support. The funding from INDEPHT is acknowledged. Scientific and Industrial Research, India. The authors are recipient of senior research fellowship, Council of fellowship, University Grant Commission; Kausik Kesh between genetic and phenotypic variation. expression may provide better understanding for links how genetic variation impacts the regulation of gene protein abundance (Battle et al., 2015). Understanding expression at all levels from mRNA to steady-state and MMP9 plays major role in both of these. A substantial metastasis are critical determinants of cancer morbidity to bind when C allele is replaced by T allele and induce promoter transcriptionally less active, thus no longer able presence of C allele at the position -1562 and keeps the protein which generally binds to the MMP9 promoter in of MMP9 in a particular locality. A nuclear repressor C/T could be an important SNP for increased expression risk more accurately for an individual. MMP9 –1562 with other genetic lesion can predict the gastric cancer might help in personalized medicine as a particular SNP well. Better understanding of SNPs in MMP9 promoter with more targeted therapies will continue to increase as knowledge to individualize therapy for cancer patients type of study. As our understanding of SNPs in MMP9 but the selection of SNPs should be appropriate for such of identifying susceptibility genes of complex disease, but the selection of SNPs should be appropriate for such type of study. As our understanding of SNPs in MMP9 promoter broadens, the opportunities to apply that knowledge to individualize therapy for cancer patients with more targeted therapies will continue to increase as well. Better understanding of SNPs in MMP9 promoter might help in personalized medicine as a particular SNP with other genetic lesion can predict the gastric cancer risk more accurately for an individual. MMP9 –1562 C/T could be an important SNP for increased expression of MMP9 in a particular locality. A nuclear repressor protein which generally binds to the MMP9 promoter in presence of C allele at the position -1562 and keeps the promoter transcriptionally less active, thus no longer able to bind when C allele is replaced by T allele and induce high MMP9 promoter activity (Figure 4). Invasion and metastasis are critical determinants of cancer morbidity and MMP9 plays major role in both of these. A substantial fraction of regulatory genetic variants influence gene expression at all levels from mRNA to steady-state protein abundance (Battle et al., 2015). Understanding how genetic variation impacts the regulation of gene expression may provide better understanding for links between genetic and phenotypic variation.

Conclusions

The SNP based study provides a promising aspect of identifying susceptibility genes of complex disease, but the selection of SNPs should be appropriate for such type of study. As our understanding of SNPs in MMP9 promoter broadens, the opportunities to apply that knowledge to individualize therapy for cancer patients with more targeted therapies will continue to increase as well. Better understanding of SNPs in MMP9 promoter might help in personalized medicine as a particular SNP with other genetic lesion can predict the gastric cancer risk more accurately for an individual. MMP9 –1562 C/T could be an important SNP for increased expression of MMP9 in a particular locality. A nuclear repressor protein which generally binds to the MMP9 promoter in presence of C allele at the position -1562 and keeps the promoter transcriptionally less active, thus no longer able to bind when C allele is replaced by T allele and induce high MMP9 promoter activity (Figure 4). Invasion and metastasis are critical determinants of cancer morbidity and MMP9 plays major role in both of these. A substantial fraction of regulatory genetic variants influence gene expression at all levels from mRNA to steady-state protein abundance (Battle et al., 2015). Understanding how genetic variation impacts the regulation of gene expression may provide better understanding for links between genetic and phenotypic variation.

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