Metalloproteinases In Medicine

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REVIEW

Matrix metalloproteinases and their inhibitors in the gastrointestinal cancers: current knowledge and clinical potential

This article was published in the following Dove Press journal:
Metalloproteinases In Medicine
2 August 2014
Number of times this article has been viewed

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Abstract: The matrix metalloproteinases (MMPs) family can degrade various components of the extracellular matrix and are implicated in a number of key normal processes. Aberrantly enhanced MMP proteolysis can lead to tumor growth, progression, and metastasis. Therefore, MMPs are considered important therapeutic and diagnostic targets for the treatment and detection of human cancers. This review summarizes the recent literature on MMPs and their inhibitors as diagnostic and prognostic predictors of gastrointestinal cancers, including esophageal squamous cell carcinoma, gastric cancer, colorectal cancer, hepatocellular carcinoma, and pancreatic cancer. Genetic and epigenetic alterations contribute to cancer progression and influence cancer susceptibility. Single nucleotide polymorphisms are the most common type of genetic variation, and can alter the expression and function of the encoded proteins. MicroRNAs are a family of small non-coding RNA molecules that function in post-transcriptional gene regulation. This review also focuses on the contribution of single nucleotide polymorphisms and microRNAs to the alteration of MMPs and their inhibitors.

Keywords: MMP, TIMP, gastrointestinal cancers, biomarker, SNP, miRNA

Introduction

The matrix metalloproteinases (MMPs) family is composed of several zinc-dependent enzymes that degrade proteins of the extracellular matrix. MMPs also regulate cell growth, survival, and migration by releasing several proteins such as growth factors, chemokines, and adhesion molecules. MMPs participate in many normal biological processes, such as embryonic development, organ morphogenesis, bone remodeling, and wound healing.[1,2] Alteration of MMP expression and activation generally promotes hallmarks of tumor progression including angiogenesis, invasion, and metastasis, and correlates with shortened survival in a variety of different cancers.[3–5]

Common structures of MMPs include a pro-peptide, a catalytic domain, a hinge region, and a C-terminal hemopexin domain. According to structure and substrate specificity, the MMP family is classified into collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), matrilysins (MMP-7 and MMP-26), membrane type MMPs (MMP-14, MMP-15, MMP-16, MMP-24, MMP-17, and MMP-25), and others (MMP-12, MMP-19, MMP-20, MMP-21, MMP-23, MMP-27, and MMP-28).[6,7]

MMPs are inhibited by endogenous tissue inhibitors of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors: TIMP-1, TIMP-2, TIMP-3, and TIMP-4.[8] These TIMPs can principally modulate MMP activity and suppress the extracellular matrix turnover by forming 1:1 enzyme/inhibitor complexes.[9] Disruption

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of this MMP/TIMP balance can result in tumor growth and metastasis. TIMP-1, TIMP-2, and TIMP-4 are present in soluble forms, while TIMP-3 is tightly bound to the matrix. TIMP-1 is the prototypic inhibitor for most of the MMP family members, but it is a poor inhibitor of the membrane type MMPs and MMP-19.[10,11] TIMP-2 is unique in that it selectively interacts with membrane type 1 MMP to facilitate the cell–surface activation of pro-MMP-2.[10–12] Thus, TIMP-2 can serve to inhibit MMP activity and to promote cell surface activation of pro-MMP-2 by membrane type 1 MMP. TIMP-3 inhibits members of the ADAM family of proteases, although the mechanism for this inhibition appears to be distinct from that of MMP inhibition.[13,14] The expression pattern of TIMP-4 seems to be distinguished from the other TIMPs. Because *TIMP-4* messenger RNAs (mRNAs) are localized in the brain, heart of adult humans, ovaries, and skeletal muscle, it may be an important tissue-specific regulator of extracellular matrix remodeling.[15,16]

MMPs and TIMPs in gastrointestinal cancers

Esophageal squamous cell carcinoma (ESCC)

Increased levels of MMP-1–3, MMP-7, MMP-9–13, MMP-15, and TIMP-2–4 have been reported in the blood or tissue samples from ESCC patients.[5,17–25] Overexpression of MMP-1 and MMP-2 in malignant tissues was significantly associated with depth of tumor invasion, lymph node metastasis, and clinical stage.[17,21,22] Patients with higher MMP-1 expression had poorer disease-free survival.[21,22] Immunohistochemical staining showed that high level MMP-9 expression in ESCC tissues correlated with tumor differentiation and lymph node status.[23] Gelatin zymography showed that the activated form of MMP-3 and MMP-10 was more strongly expressed in tumors than in paired normal tissue. Moreover, *MMP-3* and *MMP-10* mRNA levels were higher in tumors than paired normal tissues for each compartment.[19] Upregulation of MMP-10 was significantly associated with poorer disease-specific survival in early-stage ESCC (TNM stage I–IIA). In addition, multivariate analysis found that MMP-10 expression in tumor tissues was evaluated as a potential independent prognostic factor for early-stage ESCC patients.[20] MMP-13 was strongly correlated in patients who had lymph node metastasis. Cell models indicated that *MMP-13* expression was regulated by MUC1 through the Runx-2-binding site.[26] The intensity of immunohistochemical staining of MMP-15 significantly positively correlated to the intratumoral angiogenesis of ESCC. In addition, MMP-15 immunochemical intensities significantly correlated to tumor size.[27] TIMP-3 expression was significantly lower in ESCC tissues than in matched normal mucosa.[25,28] Serum levels of MMP-3, MMP-2, MMP-7, MMP-9, MMP-13, and TIMP-2 were examined in a number of studies. A study that included 53 ESCC patients and 92 healthy controls showed that serum level of TIMP-2 in the ESCC patients was significantly higher than in the control group, and was associated with patients' survival.[29] Another study revealed that patients with ESCC had significantly higher serum MMP-3, MMP-7, and MMP-9 titers than the healthy controls. Based on the optimal cutoff values for MMPs by a receiver operating characteristic, the elevated MMP-3 and MMP-9, but not MMP-7, correlated with distant metastasis and poor survival.[5] The serum level of MMP-13 was measured in 112 healthy controls and 141 ESCC patients and it was revealed that patients with an elevated level of MMP-13 ($\geq$76.4 ng/mL) had a significantly lower 5-year survival rate than those with non-elevated MMP-13 ($<$76.4 ng/mL) (Table 1).[24]

Gastric cancer (GC)

In GC, increased levels of MMP-1–3, MMP-7, MMP-9, MMP-12, MMP-14, MMP-21, MMP-24, MMP-25, and TIMP-1–3 have been reported in blood or tissue samples.[30–34] Tissue levels of MMP-3, MMP-7, MMP-12, MMP-14, and TIMP-3 were examined by using immunohistochemical staining. The expression of TIMP-3 was significantly higher, whereas that of MMP-3 and MMP-3/TIMP-3 was lower, in GC tissue of an early-stage group compared with an advanced-stage group.[30] In intramucosal cancers, *cag* pathogenicity island-dependent MMP-7 upregulation was associated with carcinogenesis.[35] MMP-12 expression was increased in GC compared with that observed in normal tissues. Increased MMP-12 and MMP-21 expression was associated with tumor invasion, distant metastasis, and TNM stage. Moreover, GC patients with MMP-12-positive expression or higher MMP-21 tended to have worse overall survival.[32,36] MMP-14 expression correlated with small tumor size, tumor at distal stomach, and increased recurrence risk. MMP-14-positive expression was a risk factor related to poor prognosis.[37] Serum levels of MMP-1, MMP-3, MMP-7, and TIMP-1 were higher in GC patients than in the healthy controls.[3,38] Serum MMP-1 and TIMP-1 levels were positively associated with morphological appearance, tumor size, depth of wall invasion, lymph node metastasis, liver metastasis, perineural invasion, and pathological stage.[38] Concomitant elevated MMP-3 ($>$14 ng/mL) and MMP-7 ($>$4.5 ng/mL)

Table 1 Expression of MMPs and TIMPs in gastrointestinal cancers

MMPs/TIMPs	Sample type	Method of analysis	Correlation
Esophageal squamous cell carcinoma			
MMP-1	Tissue[21,22,104]	IHC;[21,22] M[22,104]	Regional lymph node;[21] TNM stage;[21] poor survival[21,22]
MMP-2	Tissue;[17] serum[29]	IHC;[17] ELISA[29]	Tumor invasion depth;[17] tumor-node-metastasis stages;[17] lymph node metastasis[17]
MMP-3	Serum;[5] tissue[19,104]	Z;[19] qRT-PCR;[19] M[104]	Poor survival[5]
MMP-7			
MMP-9	Serum;[5,18] tissue[17,23,104]	ELISA;[5,18] IHC;[17,23] M[104]	Differentiation;[23] lymph node metastasis;[23] poor survival[5,23]
MMP-10	Tissue[19,20,104]	Z;[18] qRT-PCR;[19,20] IHC;[19,20] M[104]	Lymph node metastasis;[20] TNM stage;[20] poor survival[20]
MMP-11	Tissue[104]	M[104]	
MMP-12	Tissue[104]	M[104]	
MMP-13	Serum;[24] tissue[26,104]	IHC;[26] ELISA;[24] M[104]	Lymph node metastasis;[26] poor survival[24]
MMP-14	Tissue[87]	IHC[87]	Depth of wall invasion;[87] lymph node metastasis;[87] tumor stage;[87] survival;[87] recurrence[87]
MMP-15	Tissue[27]	IHC[27]	Tumor size[27]
TIMP-2	Serum[29]	ELISA[29]	
TIMP-3	Tissue[25]	IHC[25]	
TIMP-4	Tissue[25]	IHC[25]	
Gastric cancer			
MMP-1	Tissue;[31] serum[38]	RT-PCR;[31] IHC;[31] ELISA[38]	Tumor size;[38] depth of wall invasion;[38] lymph node metastasis;[31,38] liver metastasis;[38] advanced stage[38]
MMP-2	Tissue;[33] serum[39]	qRT-PCR;[33] ELISA[39]	Advanced-stage[33]
MMP-3	Tissue;[30] serum[3]	IHC;[30] ELISA[3]	Invasion;[30] lymph node invasion;[3] metastasis;[30] advanced-stage;[30] poor survival[3]
MMP-7	Peritoneal lavage fluid;[34] tissue;[35] serum[3]	qRT-PCR;[34] IHC;[35] ELISA[3]	Lymph node invasion;[3] poor survival;[3] recurrence[34]
MMP-12	Tissue[32]	IHC[32]	Tumor invasion;[32] lymph node metastasis;[32] distant metastasis;[32] TNM stage;[32] poor survival[32]
MMP-21	Tissue[36]	IHC[36]	Invasion;[36] metastasis;[36] poor survival[36]
MMP-24	Tissue[33]	qRT-PCR[33]	Advanced stage[33]
MMP-25	Tissue[33]	qRT-PCR[33]	Advanced stage[33]
MMP-14	Tissue[33,37]	IHC;[37] WB[33]	Advanced stage;[33] tumor size;[37] increased recurrence risk;[37] poor survival[37]
TIMP-1	Serum[38]	ELISA[38]	Tumor size;[38] depth of wall invasion;[38] lymph node metastasis;[38] liver metastasis;[38] advanced stage[38]
TIMP-2	Serum[39]	ELISA[39]	Depth of wall invasion[39]
TIMP-3	Tissue[30,33]	IHC;[30] WB[33]	Advanced stage;[30,33] invasion;[30] metastasis[30]
Colorectal cancer			
MMP-1	Tissue[44]	IHC[44]	Poor survival[44]
MMP-2	Tissue[43,44,105]	Z;[105] ELISA[43]	Cancer presence;[105] poor survival[43]
MMP-7	Tissue[106]	IHC[106]	Poor prognosis[106]
MMP-8	Serum[45]	TR-IFMA[45]	Tumor stage;[45] distant metastases[45]
MMP-9	Tissue[43,44,107]	IHC;[107] ELISA[43]	Recurrence;[107] poor survival[43]
MMP-13	Tissue[41]	qRT-PCR[41]	Liver metastasis[41]
MMP-21	Tissue[42]	IHC[42]	Poor prognosis[42]
MMP-14	Tissue[40]	qRT-PCR[40]	Survival[40]
TIMP-1	Tissue;[108] serum[46]	IHC;[108] ELISA[46]	Poor prognosis;[108] tumor stage; survival[46]
Hepatocellular carcinoma			
MMP-1	Tissue[49]	IHC[49]	Recurrence;[49] poorer survival[49]
MMP-2	Tissue[47]	IHC[47]	Lymph node metastasis[47]
MMP-8	Serum[51]	TR-IFMA[51]	Survival[51]
MMP-9	Tissue;[48] serum[51]	IHC;[48] ELISA[51]	Recurrence;[48] survival[48,51]
MMP-12	Tissue[51]	qRT-PCR[51]	Recurrence;[51] survival[51]
TIMP-1	Serum[51]	ELISA[51]	Survival[51]
Pancreatic cancer			
MMP-2	Tissue[109]	Z[109]	
MMP-9	Tissue[109]	Z[109]	High tumor grades[109]
TIMP-1	Serum[52]	ELISA[52]	Early stage[52]

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IHC, immunohistochemistry; M, microarray; MMP, matrix metalloproteinase; RT-PCR, reverse transcription polymerase chain reaction; q, quantitative; TIMP, tissue inhibitor of metalloproteinase; TR-IFMA: time-resolved immunofluorometric assay; WB, Western blot; Z, zymogram.

independently correlated with lymph node invasion and are predictive of shorter 2- or 5-year survivals.[3] Inversely, serum levels of MMP-2 and TIMP-2 were lower in GC patients than in healthy subjects. Concentrations of TIMP-2 and carcinoembryonic antigen correlated with depth of wall invasion (Table 1).[39]

Colorectal cancer (CRC)

MMP-1, MMP-2, MMP-7, MMP-9, MMP-13, MMP-14, MMP-21, and TIMP-1 expression in CRC were examined.[40–44] Levels of MMP-1 in tumor-free mucosa tissue were significantly associated with cancer-specific survival in CRC in univariate analysis. This prognostic strength was maintained for MMP-1 and N-status in multivariate analysis.[44] A high protein expression of MMP-2 as well as MMP-9 in normal mucosa was found to correlate with worse 5-year survival.[43] Expression levels of *MMP-7*, *MMP-9, MMP-13*, and *TIMP-1* were higher in cancer tissue than in adjacent normal mucosa. *MMP-13* expression correlated with liver metastasis.[41] *MMP-14* gene expression was higher in cancer tissue than in adjacent normal mucosa. Overall 5-year survival differed significantly between patients with high *MMP-14* gene expression and those with low expression.[40] MMP-21 expression was significantly higher in CRC as compared with in normal epithelial tissue, and correlated with tumor invasion, lymph node metastasis, and distant metastasis. Moreover, MMP-21 was also proven to be an independent prognostic factor in patients with stage II as well as stage III CRC.[42] A study of 48 CRC patients and 83 healthy controls showed that serum levels of MMP-8 were higher in the patients than those in the controls and positively correlated with disease stage, the degree of primary tumor necrosis, and blood neutrophil count.[45] Serum MMP-9 and TIMP-1 were measured and significantly higher in CRC patients than in healthy subjects. Concentrations of TIMP-1 correlated with tumor stage, nodal involvement, presence of distant metastases, and patient survival (Table 1).[46]

Hepatocellular carcinoma (HCC)

Protein or mRNA levels of *MMP-1, MMP-2, MMP-9*, and *MMP-12* were examined in HCC subjects.[47–49] MMP-1 and PAR-1 levels were high in HCC tissues compared with normal liver tissues. The overexpression of MMP-1 and PAR-1 was significantly associated with recurrence, TNM staging, and portal vein invasion of HCC as well as significantly poorer overall survival and disease-free survival.[49] Upregulated MMP-9 was associated with both time to recurrence and overall survival.[48] *MMP-12* mRNA was significantly higher in HCC tissues than in non-tumor and normal liver tissues. Overexpression of *MMP-12* mRNA significantly correlated with presence of venous infiltration, early tumor recurrence, and poor overall survival of HCC patients.[50] Patients with higher concentrations of serum MMP-8 or TIMP-1 had a significantly worse overall survival than patients with low concentrations of serum MMP-8 or TIMP-1. The overall survival in patients with a high ratio of MMP-9/TIMP-1 was statistically significantly better than in those patients with a low ratio of MMP-9/TIMP-1 (Table 1).[51]

Pancreatic cancer

Serum level of TIMP-1 was higher in pancreatic ductal adenocarcinoma than in chronic pancreatitis or healthy control subjects, and was correlated with reduced patient survival (Table 1).[52]

Genetic and epigenetic regulation of MMPs and TIMPs

Carcinogenesis is a complex process during which cells undergo genetic and epigenetic alterations. Genetic polymorphism is an important determinant of endogenous causes of cancer. Single nucleotide polymorphism (SNP) is the most common type of genetic variation. SNPs can alter the expression and function of the encoded proteins. A number of SNPs have been identified in the genes encoding MMPs and TIMPs. These SNPs have been associated with susceptibility to some diseases, including gastrointestinal cancers as summarized in Table 2.

Epigenetic alterations can lead to modified gene expression, including DNA methylation, histone modification, and microRNAs (miRNAs). miRNAs have become increasingly important to gene regulation. miRNAs are small non-coding RNAs of 18–25 nucleotides that negatively regulate gene expression through base pair matching with the 3′-untranslated region of their target mRNAs and resulting translation repression or degradation.[53,54] Many studies have verified that miRNAs regulate cell proliferation, cell invasion, and cell apoptosis.[55–57] Many miRNAs may function as both oncogenes and tumor suppressors, and abnormal expression of miRNA is associated with a variety of human cancers. A growing number of miRNAs have been identified to regulate MMP or TIMP expression directly or indirectly via other regulatory factors, resulting in a disruption of distinct MMP functions. These functions are involved in several of the processes – such as extracellular matrix remodeling, epithelial to mesenchymal transition, and angiogenesis – that

support cancer progression. In gastrointestinal cancers, some recent data are summarized in Table 3.

SNPs of MMPs and TIMPs and gastrointestinal cancer susceptibility

The *MMP-1* promoter region at position −1607 contains 1G and 2G SNPs relative to the transcriptional start site. The insertion of an additional G results in an ETS-1 binding site, and it has been shown that the 2G allele has higher transcriptional activity compared with the 1G allele.[58] In a study of an Iranian population with CRC, individuals with the 2G/2G genotype seemed to spread metastasis 3 years earlier than those who were 1G/1G and 1G/2G.[59] In a Chinese population, there were no significant differences in the genotype and allele frequency of the *MMP-1* −1607 1G/2G variant between the case group (n=237) and healthy controls (n=252).[60] Another study of Indian cases with GC (n=145) and healthy controls (n=145) demonstrated that the *MMP-1* −422T>A SNP showed significant risk for regional lymph node metastasis. In addition, the *MMP-1* −519A>G SNP displayed poor cellular differentiation, attributing to a higher risk of cancer progression.[61]

Two SNPs in the −1306C>T and −735C>T *MMP-2* promoter region may influence MMP-2 expression. In vitro experiments showed that the −1306T and −735T alleles had lower promoter activity than the −1306C and −735C alleles, resulting from C to T transitions that abolished Sp1 binding.[62] These two SNPs were associated with *MMP-2* polymorphisms and ESCC in a Chinese population. Only the allele and genotype distributions of the *MMP-2* −1306C>T SNP had significant differences between patients with ESCC and healthy controls. Individuals with the −1306 C/C genotype had a significantly increased risk of ESCC, especially those who smoked or had positive family history.[63] The *MMP-2* −1036C>T SNP

Table 2 SNPs of MMPs and TIMPs in gastrointestinal cancers

Gene	SNP (rs number)	Cancer type	Ethnicity	Comments
MMP-1	−422T>A (rs475007)	GC	Indian[61]	T carrier showed significant risk for regional lymph node metastasis
	−519A>G (rs1144393)	GC	Indian[61]	G carrier displayed poor cellular differentiation attributing to a higher risk of cancer progression
	−1607 1G>2G (rs11292517)	CRC	Iranian[59]	2G/2G genotype with invasion risk of CRC
MMP-2	−1306C>T (rs243865)	ESCC	Chinese[63]	C/C genotype correlated with increased risk of ESCC
		CRC	Netherlander[64]	T/T genotype was associated with poor survival
	−1575G>A (rs243866)	CRC	Taiwanese[67]	A/A genotype had a 6.32-fold higher risk of developing distant metastasis
MMP-3	−1612 5A>6A (rs35068180)	HCC	Japanese[69]	5A carriers had a significantly poorer prognosis
MMP-7	−181A>G (rs11568818)	ESCC	Indian[72]	G/G genotype carries a higher risk of ESCC
		GC	Indian[73]	G/G genotype was associated with a more than two-fold increased risk of GC
		GC	Japanese[70]	G carrier associated with an increased risk for *Helicobacter pylori*-related noncardial GC
		CRC	Pole[71]	G/G had an increased risk of CRC, higher lymph node involvement, and advanced tumor infiltration
MMP-9	P574R (rs2250889)	ESCC	Chinese[79]	G/G genotype was associated with a significantly increased risk of ESCC
		GC	Chinese[80]	P/P associated with lymph node metastasis
	R279Q (rs17576)	GC	Chinese[80]	R/R associated with lymph node metastasis
		CRC	Chinese[60]	R/R associated with an increased risk of CRC
	−1562C>T (rs34016235)	CRC	Korean[82]	C/C associated with higher risk of CRC
MMP-12	−82A>G (rs2276109)	CRC	Swedish[85]	A/A genotype is connected with a higher risk of disseminated CRC
MMP-14	6767G>A (rs1042704)	HCC	Taiwanese[86]	G/G genotypes had higher risk of HCC
	7096T>C (rs2236307)	HCC	Taiwanese[86]	T/T genotypes had higher risk of HCC
TIMP-2	303C>T (rs2277698)	GC	German[65]	C/C genotype associated with higher lymph node metastasis and more distant metastasis
		CRC	Korean[82]	C/C associated with higher risk of CRC
	−418G>C (rs8179090)	CRC	Korean[82]	G/G associated with higher risk of CRC and metastasis

Abbreviations: CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; GS, gastric cancer; HCC, hepatocellular carcinoma; MMP, matrix metalloproteinase; PC, pancreatic cancer; rs, reference single nucleotide polymorphism cluster identification; SNP, single nucleotide polymorphism; TIMP, tissue inhibitor of metalloproteinase.

was examined in a Dutch population with CRC. Individuals with the *MMP-2* −1036 T/T genotype were associated with poor survival as compared with the C/C and C/T genotypes. Higher MMP-2 expression in tumors also correlated with poor prognosis. However, MMP-2 protein levels in CRC homogenates did not correlate with their respective SNP genotypes.[64] Studies of a Korean population with CRC and a German population with GC showed that there were no significant differences in the genotype distributions and allele frequencies of *MMP-2* −1036C>T between cases and healthy controls.[65,66] A cohort of 282 CRC patients in a Taiwanese population was examined for *MMP-2* SNPs, including −1575G>A and −375C>T. Patients carrying the A/A genotype had a 6.32-fold higher risk of developing distant metastasis.[67]

The promoter region of *MMP-3* located at nucleotide position −1612 is characterized by a 5A/6A polymorphism relative to the transcriptional start site, with one allele having 6A and the other having 5A at the polymorphic site. In vitro experiments showed that the 5A allele has greater promoter activity than the 6A allele.[68] In a Japanese population with HCC, it was shown that *MMP-3* −1612 5A carriers had significantly larger HCC diameters and poorer prognosis than *MMP-3* −1612 6A homozygotes.[69]

The susceptibility of the *MMP-7* −181A>G SNP to cancer was examined in several studies.[70–73] Functional analysis has shown that the promoter activity of the −181G allele was two- to three-fold higher than that of the −181A allele.[74] Computer analysis revealed that the −181G site coincides with a putative binding site (NGAAN) for a heat shock transcription factor, which is not present in the −181A allele.[74,75] Individuals with the *MMP-7* −181G/G genotype had a higher risk of ESCC in an Indian population.[72] Two studies reported on the *MMP-7* −181A>G SNP in relation to GC susceptibility. A study of 108 GC patients and 195 healthy controls in an Indian population showed that the G/G variant genotype of the *MMP-7* −181A>G SNP was associated with a more than two-fold increased risk of GC compared with the common A/A genotype. Smoking or a high intake of salted tea was not influenced by the *MMP-7* polymorphism.[73] Similarly, a study from a Japanese population showed that the *MMP-7* −181G allele carrier relative to the A/A genotype significantly increased the risk of *Helicobacter pylori* related GC, especially in patients with noncardial cancer.[70] A study of 184 Polish CRC patients showed that the G/G genotype had an increased risk of CRC and higher lymph node involvement than the A/A genotype. Moreover, the G/G genotype was associated with advanced tumor infiltration.[71] In a German population, it was shown that there were no significant differences in the genotype distributions and allele frequencies of *MMP-7* −181G>C between GC patients and healthy controls.[65]

There is evidence indicating that the −1562C>T SNP in the *MMP-9* gene promoter has an effect on *MMP-9* expression, with the T allele having 1.5-fold higher promoter activity than the C allele.[76] In the coding region of the *MMP-9* gene, non-synonymous SNP R279Q (G to A) at exon 6 may cause the amino acid change required for binding to its substrate and affecting its binding ability.[77] In addition, non-synonymous SNP P574R at exon 10 (C to G) has been shown to be functional in lung cancer.[78] A Chinese study showed the P574R G/G genotype was associated with a significantly increased risk of ESCC compared with the C/C genotype.[79] In a Chinese population, the homozygous *MMP-9* 279R/R and 574P/P genotypes were associated with lymph node metastasis, but not with the occurrence of GC.[80] Also in a Chinese population, the R/R genotype of *MMP-9* 279Q>R was associated with a 2.2-fold increased risk of CRC as compared with the QQ genotype.[60] However, an inconsistent result from another Chinese study showed that P574R and R668Q of *MMP-9* were not associated with CRC risk.[81] In Korea, the homozygous *MMP-9* −1562 C/C genotype was significantly more frequent in CRC patients than in healthy controls.[82]

The −82A>G SNP at in the promoter of the *MMP-12* gene is located at a core recognition sequence of AP-1. In vitro experiments showed that the A allele increased the binding ability of AP-1 to enhance the gene transcription.[83] Functional analysis of *MMP-13* −77A>G SNP may lead to alterations in the gene expression.[84] A Swedish study showed that the *MMP-12* −8 1A/A genotype, but not *MMP-13* genotypes, was connected with a higher risk of disseminated CRC.[85]

The *MMP-14* SNPs, including −165T>G, 221T>C, 6727C>G, 6767G>A, 7096T>C, and 8153G>A have been validated to correlate with risk of HCC in Taiwan. Individuals with the *MMP-14* 6767 G/A or 7096 C/C genotype exhibited a significantly lower risk of HCC.[86] There was no significant difference in the genotype distributions and allele frequencies of *TIMP-2* 303C>T between GC patients and controls in a German population. The correlations between the determined SNPs and clinicopathological parameters showed that patients with the CC genotype had significantly higher lymph node metastasis and more distant metastasis.[65] In CRC, two SNPs at promoter −418 and −303 of *TMP-2* were assayed in a Korean population. The homozygous *TIMP-2* −418 G/G and *TIMP-2* −303 G/G genotypes were significantly more frequent in CRC patients than in healthy controls. Individuals with the *TIMP-2* −418 C/C genotype were more frequent in

CRC patients with a family history of cancer. The frequency of the *TIMP-2* –418 G/G genotype was significantly higher in CRC patients with distant metastasis than in those without distant metastasis.[82]

miRNA regulation of MMPs and TIMPs

In ESCC, MMP-14 has been identified as an independent poor prognostic factor. A bioinformatic analysis identified one conserved sequence site for miR-133a in the 3′-untranslated region of *MMP-14*. The expression levels of miR-133a were significantly lower in the tumor tissues than in the normal tissues. *MMP-14* mRNA levels in surgical specimens significantly inversely correlated with miR-133a. Transfection of the miR-133a inhibitor significantly increased *MMP-14* mRNA levels. Knockdown of *MMP-14* or transfection of an miR-133a mimic inhibits the proliferation and invasion of ESCC cells.[87] miR-21 downregulation can inhibit the metastasis of ESCC by targeting *TIMP-3*.[88]

In GC, downregulation of miR-148a GC tissues was reported by using an mRNA microarray. In oligonucleotide array analysis, *MMP-7* was markedly downregulated in miR-148a-overexpressing GC cells. *MMP-7* was found to be a direct and functional target of miR-148a, participating in cell invasion.[89] MiR-145 suppressed the invasion–metastasis cascade in GC by directly targeting N-cadherin protein translation and then indirectly downregulated its downstream effector MMP-9.[90] *TIMP-2* was found to be the target of miR-106a. Upregulated miR-106a positively adjusted GC cell proliferation, migration, and invasion. Knockdown of *TIMP-2* could mimic the miR-106a-induced cancer development benefits. Inverse correlation of miR-106a and *TIMP-2* ultimately contributed to the enhancement of GC progression.[91]

Three reports have implicated miRNAs in the direct or indirect regulation of *MMP-2* in CRC. It was found that high expression of miR-29a and miR-29b promoted cancer metastasis by upregulation of MMP-2 through targeting KLF4 in colorectal tumorigenesis.[92,93] In CRC, decreased miR-139 was found to be associated with invasion and metastasis. Downregulation of miR-139 promotes migration inactivation of CRC cells by increasing MMP-2 through targeting type I IGF-IR and regulating downstream MEK/ERK signaling.[94] Downregulation of let-7c in cancer tissues was significantly associated with metastases, advanced TNM stage, and poor survival of CRC patients.[95]

Downregulation of miR-29b was associated with poor recurrence-free survival of HCC patients. In human HCC tissues and mouse xenograft tumors, miR-29b levels were inversely correlated with MMP-2 expression as well as tumor angiogenesis, venous invasion, and metastasis.[96] Through miRNA microarray analysis, miR-491 level was significantly downregulated in poorly differentiated HCC tissue compared with well-differentiated HCC tissue. miR-491 levels were also inversely correlated with different status of differentiation in HCC tissues and with migratory potential in HCC cell lines.[97] miR-491 was predicted to bind the *MMP-9* mRNA 3′-untranslated region via TargetScan (http://www.targetscan.org/) and microRNA.org (http://www.microrna.org/microrna/home.do) algorithms. A study of glioblastoma multiforme suggests that miR-491 is the *MMP-9*-specific miRNA, which downregulates *MMP-9* mRNA directly.[98] miR-125a was frequently downregulated in HCC compared with matched adjacent liver tissues, and correlated with the malignant progression of patients. miR-125a inhibits the proliferation and metastasis of HCC by targeting *MMP-11*.[99] *TIMP-3* was identified as a target of miR-181b, miR-181d, miR-221, and miR-222.[100,101] In a diet-induced HCC mouse model, miR-181b and miR-181d levels were upregulated. mir-181b target *TIMP-3* mRNA leads to downregulation of TIMP-3 level and subsequent

Table 3 MMPs and TIMPs are targeted directly or indirectly by miRNAs in gastrointestinal cancers

MMPs/ TIMPs	miRNAs	Cancer type	Target
MMP-2	miR-29a	CRC[93]	Target KLF4, and then indirectly downregulate its downstream MMP-2
	miR-29b	CRC;[44] HCC[45]	Target *MMP-2* directly
	miR-139	CRC[94]	Target IGF-IR, and then indirectly downregulate its downstream MMP-2 via IGF-IR/MEK/ERK signaling
MMP-7	miR-148a	GC[89]	Target *MMP-7* directly
MMP-9	miR-145	GC[90]	Target N-cadherin, and then indirectly downregulate its downstream MMP-9
	miR-491	HCC[97]	Target *MMP-9* directly
MMP-11	miR-125a	HCC[99]	Target *MMP-11* directly
	let-7c	CRC[95]	Target *MMP-11* directly
MMP-14	miR-133a	ESCC[87]	Target *MMP-14* directly
	let-7	PC[102]	Target MMP-14 indirectly
TIMP-2	miR-106a	GC;[53] PC[54]	Target *TIMP-2* directly
TIMP-3	miR-21	ESCC[88]	Target *TIMP-3* directly
	miR-181b,	HCC[101]	Target *TIMP-3* directly
	miR-181d, miR-211, miR-222	HCC[100]	Target *TIMP-3* directly

Abbreviations: CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; GS, gastric cancer; HCC, hepatocellular carcinoma; miRNA, microRNA; MMP, matrix metalloproteinase; PC, pancreatic cancer; TIMP, tissue inhibitor of metalloproteinase.

enhancement of MMP-2 and MMP-9 activities, promoting migration and invasion.[101] miR-221 and miR-222 were found to be increased in HCC to target *TIMP-3* and *PTEN* due to the activation of MMP-3, MMP-9, and the AKT pathway.[100]

In pancreatic ductal adenocarcinoma, MMP-14 expression significantly correlated with decreased levels of let-7 in human pancreatic ductal adenocarcinoma tumor specimens. Let-7 indirectly regulates the expression of MMP-14 and ERK1/2 activation.[102] In addition, a high level of miR-106a expression has an oncogenic role in pancreatic tumorigenesis by targeting *TIMP-2*.[103]

Clinical potential

The imbalance between MMPs and TIMPs can facilitate the early carcinogenesis, tumor development, growth, invasion, and metastasis of cancer cells. Alternative expressions of MMPs and TIMPs have been found in ESCC, GC, CRC, HCC, and even pancreatic cancer. It indicates that the concentrations of various MMPs and TIMPs are upregulated in the tissue, plasma, or even serum of cancer patients and may thus correlate with the tumor stage, depth of tumor invasion, presence of nodal, distant metastases, and patient survival. Therefore, these MMPs and TIMPs can be applied as diagnostic or prognostic markers for these cancers. The control of MMP activity may thus be generated as a potential therapeutic target to inhibit tumor progression. SNP analysis also offers the potential for a better diagnosis of various diseases and prediction of clinical outcome and therapeutic efficiency in several cancers. To predict the cancer risk in a population and the outcome, several genes would probably be a better tool than the use of a single SNP. Alternative expressions of miRNAs are also disclosed in a variety of cancers. MMP and TIMP levels are exactly regulated by miRNAs in either a direct or indirect manner. Utilization of miRNA mimics may thus provide inhibition of cancer progression via targeting the related MMPs or their upstream regulators.

Disclosure

The authors report no conflicts of interest in this work.

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