The Role of Matrix Metalloproteinases in Stromal/Epithelial Interactions in the Gut

The gastrointestinal mucosa is an extremely soft, highly vascularised tissue, with a single layer of epithelium separating the gut lumen from the host. Epithelial cells adhere to a thin basement membrane that is produced by both epithelial cells and the underlying stromal cells. Signals passing between epithelial cells and stromal cells are needed for normal gut structure. In gut diseases, however, epithelial cells and stromal cells produce large amounts of matrix degrading enzymes (matrix metalloproteinases), the function of which is only beginning to be elucidated. Here, we review the role of matrix metalloproteinases (MMPs) in the gut in health, gut inflammation, and in cancer.

The matrix metalloproteinases (MMPs) are metzincin proteases whose primary function is in extracellular matrix (ECM) degradation and remodelling (9, 42, 43). MMPs are mostly secreted as latent, inactive zymogens by various stromal and epithelial cell types, including mesenchymal cells, T cells, monocytes, macrophages, neutrophils, keratinocytes, and tumor cells. Activation into active enzyme usually occurs in the pericellular or extracellular space. MMP proteolysis of the extracellular matrix creates space into which cells can migrate, produces specific fragments of extracellular matrix proteins with independent biological activity, regulates tissue architecture, and activates, deactivates, and modifies the activity of signalling molecules (58).

MMPs are structurally related but can be subdivided according to their primary substrate specificities: collagenases (MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -7, -10, -11), elastase (MMP-12), membrane types (MMP-14, -15, -16, -17, -24, -25), and others (MMP-19, -20, -23, -26, -27, -28) (Table 1). MMPs work together to create a cascade of activation, whereby, once one MMP is activated, it catalyses the conversion of other MMPzymogens to their active forms so that many MMPs are “switched on” with the potential to degrade all classes of the extracellular matrix. Endogenous MMP inhibitors are the tissue inhibitors of metalloproteinases (TIMPs), produced by the same cells that produce MMPs and that act by forming 1:1 complexes with MMPs.

Under normal conditions, MMPs are present in tissues at low levels usually in the latent form and are responsible for normal physiological tissue turnover. They are regulated in four ways: at the level of transcription, at the point of activation from the precursorzymogens, by interaction with specific ECM components, and by TIMP inhibition (10; 19). TIMPs control the local activities of MMPs in tissues. However, if the production of MMPs is excessive, TIMP inhibition can be inadequate to control the flood of MMPs, leading to an imbalance in the ECM breakdown and repair system. For example, if ECM degradation is excessive and the healing response cannot maintain integrity, tissue will be digested away with structural loss, as in the fistulous tracts often seen in Crohn’s disease (27).

Normal Biology of Stromal/Epithelial Cell Interactions in the Gut

Stromal cells are classified as any nonepithelial cells involved in the architecture of the gastrointestinal tract and are a major component of the normal gut. They include mesenchymal cells such as myofibroblasts, fibroblasts, muscle cells, microvascular endothelial cells, and inflammatory cells. ECM proteins specifically form the noncellular component of the gut.

The luminal surface of the small bowel is ordered into numerous villi and crypts, covered by a continuous sheet of cells in perpetual upward motion toward the lumen. In the colon, the surface is flat with the crypts forming deep invaginations. The massive rate of cell production in the crypts is compensated by apoptosis and shedding of epithelial cells at the tip of the villus in the small bowel and from the surface of the colon. Stem cells reside near the bottom of the crypts and escape from this upward flow (49). The mucosal lamina propria is a loose and highly vascularized connective tissue matrix consisting of structural collagens, glycoproteins, chondroitin sulphate proteoglycans, hyaluronic acid, and ground substance. These are secreted by the lamina propria myofibroblasts (mesenchymal cells). Where the stromal cells abut the epithelium and endothelium, the two cell types cooperate in the construction of the basement membrane, containing laminin, heparan sulphate proteoglycans, and collagen IV. Of all the tissues in the body, the intestinal lamina propria is the most plastic, where the degradation and repair of the extracellular matrix must be tightly controlled (34).
stem cells of the small intestine, have a missing crypt progenitor compartment, implying that physiological Wnt signaling is required for its establishment (29). Inhibition of Wnt signaling by the transgenic expression of Dkk-1, involved in embryonic development through its inhibition of the Wnt signaling pathway,

Table 1. The matrix metalloproteinases and its targets of activity

<table>
<thead>
<tr>
<th>MMP</th>
<th>Common Name</th>
<th>Substrate of Enzymatic Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP1</td>
<td>Collagenase 1</td>
<td>Aggrecan, collagen I, II, III, VII, X, XI, fibronectin, laminin, link protein, tenasin, vitronectin, IL-1β, pro-TNF, CTGF</td>
</tr>
<tr>
<td>MMP2</td>
<td>Gelatinase A</td>
<td>Aggrecan, collagen I, III, IV, V, VII, X, XI, decorin, elastin fibropectin, gelatin, laminin, link protein, tenasin, vitronectin, IL-1β, pro-TGFβ, pro-TNF</td>
</tr>
<tr>
<td>MMP3</td>
<td>Stromelysin 1</td>
<td>Aggrecan, collagen III, IV, V, VII, IX, X, XI, decorin, elastin fibropectin, gelatin, laminin, link protein, tenasin, vitronectin, E-cadherin, IL-1β, pro-TNF, CTGF</td>
</tr>
<tr>
<td>MMP7</td>
<td>Matrilysin</td>
<td>Aggrecan, collagen I, IV, decorin, elastin, fibropectin, laminin, link protein, tenasin, vitronectin, E-cadherin, pro-TNF, &lt;alpha defensin, CTGF</td>
</tr>
<tr>
<td>MMP8</td>
<td>Collagenase 2</td>
<td>Aggrecan, collagen I, II, III</td>
</tr>
<tr>
<td>MMP10</td>
<td>Stromelysin 2</td>
<td>Aggrecan, collagen III, IV, V, elastin, fibropectin, gelatin, link protein, fibrinogen</td>
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<tr>
<td>MMP11</td>
<td>Stromelysin 3</td>
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</tr>
<tr>
<td>MMP12</td>
<td>Macrophage metalloelastase</td>
<td>Aggrecan, collagen I, IV, elastin, fibropectin, gelatin, laminin, vitronectin, pro-TNF</td>
</tr>
<tr>
<td>MMP13</td>
<td>Collagenase 3</td>
<td>Aggrecan, collagen I, II, III, VI, IX, X, XI, XIV, fibropectin, gelatin, CTGF</td>
</tr>
<tr>
<td>MMP14</td>
<td>MT-1 MMP</td>
<td>Aggrecan, collagen I, II, III, fibropectin, gelatin, laminin, vitronectin, pro-MMP2, pro-TNF</td>
</tr>
<tr>
<td>MMP15</td>
<td>MT2-MMP</td>
<td>Collagen III, fibropectin,</td>
</tr>
<tr>
<td>MMP18</td>
<td>Collagenase IV (Xenopus)</td>
<td>Collagen I</td>
</tr>
<tr>
<td>MMP19</td>
<td>RAS-1</td>
<td>Collagen I, IV, fibropectin, gelatin, tenasin</td>
</tr>
<tr>
<td>MMP20</td>
<td>Enamalysin</td>
<td>Unknown</td>
</tr>
<tr>
<td>MMP21</td>
<td>XMMP (Xenopus)</td>
<td>Unknown</td>
</tr>
<tr>
<td>MMP22</td>
<td>CMMP chicken</td>
<td>Unknown</td>
</tr>
<tr>
<td>MMP23</td>
<td>MT3- MMP</td>
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</tr>
<tr>
<td>MMP24</td>
<td>MT5-MMP</td>
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<td>MT6-MMP</td>
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</tr>
<tr>
<td>MMP28</td>
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also induces the complete loss of crypts, identifying Wnt as the dominant mitogen for crypt progenitor cells throughout life (49). Transgenic expression of R-spondin-1, a Wnt agonist, results in hyperproliferation of intestinal crypts (26). Wnt proteins appear to be physiologically expressed by the crypt epithelial cells rather than by the surrounding stroma, which not only stimulate the proliferation of crypt progenitors but also promote the terminal differentiation of the Paneth cells residing at the bottom of the crypts in the small bowel (FIGURE 1) (64).

The migration of T cells across the endothelial cell (EC) barrier that separates blood from tissue involves complex morphological changes in T cells and the EC barrier as well as multiple interactions between cell-cell and cell-matrix adhesion molecules. Selectins mediate the initial capture of leukocytes and the rolling phase, followed by EC-expressed chemokines inducing integrins to assume a high affinity state leading to leukocyte arrest and firm adhesion. The basement membrane represents a major barrier to migrating T cells, which involves the inducible expression of proteases, including MMP-2 and -9 (18, 35), to transit across. MMP-3 is also partly necessary for the migration of CD4+ T cells into the intestinal mucosa (31). It has recently been clearly demonstrated that Wnt signaling plays an important role in upregulating MMP expression in T cells, and EC-derived Wnts trigger upregulation of MMP-2 and -9 in effector T cells, thereby enhancing their ability to cross the subendothelial basement membrane and migrate into peripheral sites (68).

MMPs are generally involved in the recruitment of inflammatory cells into the intestinal wall. The presence of MMP activity is necessary for lymphocyte transmigration across the endothelial venules into lymph nodes (17). Although the precise role of MMP-9 in polymorphonuclear neutrophil migration across the endothelial cells into the lamina propria is unclear, MMP9 is secreted during neutrophil migration across the basement membrane, and this migration is inhibited by TIMP-1 (15). Myofibroblast MMPs are able to enhance the neutrophil chemoattractant capacity of intestinal epithelial cells by the activation of the neutrophil chemoattractant CXCL7 (30).

Matrix Metalloproteinases in Stromal/Epithelial Interactions in Disease

MMPs are likely to play a much more important role in the gut in disease where they are an important component of the inflammatory milieu. In the broadest sense, the epithelial barrier of the gut depends on adhesion of enterocytes to an intact basement membrane, and if this basement membrane and the associated growth factors are modified or destroyed by MMPs produced by either epithelial cells, stromal cells, or inflammatory cells, it is likely to have profound effects on the host.

Inflammation bowel diseases (IBD)

Ulcerative colitis and Crohn’s disease represent the two most common and serious types of idiopathic inflammatory bowel disease. Ulcerative colitis is a relapsing inflammatory disease that is restricted to the mucosa of the colon. Crohn’s disease, on the other hand, is a relapsing transmural inflammatory disease that can affect the entire gastrointestinal tract from the mouth to the anus (6). Although ulceration is a feature of many idiopathic and infectious diseases of the gut, it is only recently that MMPs have been implicated in the pathology of gut ulcers.

In situ hybridization studies show high expression of MMP-1 and MMP-3 transcripts in the granulation tissue around ulcers of patients with Crohn’s disease or UC. Fibroblasts in these ulcers express collagenase 3 (MMP-13) and TIMP-3 (63). The epithelium at the edge of ulcers is strongly positive for matrilysin (MMP-7), which plays an important role in epithelial restitution after injury (16). There is disruption of the basement membrane below the MMP-7 positive epithelium in IBD (50), suggesting that the epithelium itself may be an active participant in its own shedding. It is, however, unclear whether this is an adaptive event to allow epithelial cells to migrate faster or whether...
chimeric TNF-α antibody, is used in the treatment of inflammatory bowel disease and downregulates MMP-1, -3, and -9 relative to TIMP-1 and -2, thereby causing significant clinical improvement (39). Overall, therefore, there is strong evidence that increased MMPs, made by both epithelial cells and stromal cells, is important in gut injury (FIGURE 2).

Myofibroblast cultures established from fibrotic Crohn’s disease mucosal samples have been shown to have significantly higher constitutive expression of TIMP-1 when compared with normal or ulcerative colitis tissue (38). The same study also showed that IBD myofibroblasts also expressed MMP-1, -2, and -3 but not MMP-9. Recombinant TGF-β1 and β2 induced expression of TIMP-1 in normal intestinal myofibroblasts, illustrating a potential mechanism by which differential expression of isoforms of TGF-β may lead to excess deposition of ECM and stricture formation via TIMP-1-mediated inhibition of MMP activity (38).

Models of colitis in mice have shown that MMP-9 derived from the epithelium is crucial in the induction of intestinal damage (11). This implies that MMP-9 released from epithelial cells during inflammation is responsible for the degradation of ECM components with subsequent loss of mucosal integrity, leading to an enhanced penetration of inflammatory cells and the facilitation of cellular interactions with luminal antigens. On the other hand, other epithelial-derived MMPs may be responsible for epithelial migration on intestinal inflammation, thereby assisting in wound healing. MMP-7 has also been shown to be expressed by migrating enterocytes at the margin of intestinal ulcers in patients suffering from ischaemic colitis, suggesting that it is generally switched on when epithelial cells try to heal denuded mucosa, whatever its origin (52).

Increased concentrations of pro-inflammatory cytokines in inflamed mucosa appear to be responsible for the increase of MMPs in inflammatory diseases of the gut. Activation of lamina propria T cells in explants of human fetal gut leads to a dramatic increase of MMP-1 and MMP-3, which leads to severe tissue damage with epithelial cell shedding and loss of villi, an effect that was abolished by the use of a synthetic MMP inhibitor (46). In the same model, a p55 TNF receptor immunoadhesin prevented T-cell-mediated intestinal injury by inhibiting MMP production by mesenchymal cells, which suggests that TNF-α induces intestinal damage by the stimulation of MMP secretion (45). Activated T cells release IL-22 that can induce increased mRNA MMP expression by colonic subepithelial fibroblasts (2), and we have also recently shown that IL-21 increases MMP production by gut fibroblasts (41).

A frequent manifestation of gut inflammation in IBD is the lengthening of the crypts and increased epithelial proliferation. This may be mediated by increased production of keratinocyte growth factor (KGF) from gut stromal cells activated by proinflammatory cytokines.
KGF is overexpressed in active IBD stromal cells, and inhibition of KGF decreases T-cell-mediated crypt hyperplasia (3; 4). Crypt hyperplasia and lengthening occurs in both small bowel and colon and may have evolved as a mechanism to eliminate pathogens living inside epithelial cells or colonizing the epithelial surface. In IBD, it serves to reduce the gut absorptive surface area, and increased migration is associated with more leaky tight junctions, leading to increased fluid, electrolyte, and protein loss into the gut lumen.

**Peptic ulcers and Helicobacter pylori infection**

*H. pylori* is highly prevalent in developed nations and ubiquitous in developing countries (59). For reasons which are not fully understood, in some patients, infection is associated with gastric ulceration, together with gastric adenocarcinomas and lymphomas. MMP-1 and -3 are abundant in peptic and duodenal ulcers (63). It is also clear that MMP-1 and -7 is upregulated in the gastric epithelium in response to *H. pylori* infection in vivo and in vitro and in gastric mucosa (14), and this is dependent on an intact *cag* virulence factor (7, 47). The induction of MMP-7 by *H. pylori* plays a role in stimulating gastric epithelial cell migration requiring the adhesion of bacteria to epithelial cells and is mediated by small GTPases of the Rho family through the activation of transcription factors such as NF-κB and AP-1 (67).

Interestingly, genetic variants in MMP-7 and -9 involved in the inflammatory response to *H. pylori* could predispose patients with chronic *H. pylori* infection to develop gastric ulcer disease, with the level of association pointing to a complex genetic disease. Patients carrying the rare allele G of the MMP-7-181 polymorphism have a 1.6-fold increased risk of developing gastric ulcer, and carriage of the A allele of a coding SNP in exon 6 of MMP-9 conferred a 2.4-fold increased risk, with an increased expression of the protease (22). However, this association study was conducted irrespective of the variety of *H. pylori* strain, known to be a risk factor, and focused solely on the host genetic factors.

**Coeliac disease**

Coeliac disease (CD) is an immune disorder affecting the small bowel, which occurs in genetically predisposed individuals in all age groups after early infancy. It is caused by a Th1 cell response to the wheat, barley, and rye protein gliadin. CD is characterized by deepening of the crypts and flattening of the villi in the small intestine. Rapid collapse of the villi can occur within hours of administering gliadin to CD patients (55). MMP-1, -3, and -12 are increased in the subepithelial region of untreated CD mucosa, and they are mainly localized to subepithelial fibroblasts and macrophages, contributing to the degradation of intestinal collagen and to epithelial...
cell shedding (53). The increased expression of matrix metalloproteinases-1, -3, and -9 and the tissue inhibitor metalloproteinase-1 in duodenal mucosal biopsies from patients with gluten-sensitive enteropathy suggests a role for these enzymes in the tissue remodeling, which is a feature of this disorder (40).

Dermatitis herpetiformis is a specific dermatological manifestation of CD with small blisters and pathognomonic IgA deposits in the papillary dermis. It has been shown that there was a big increase in activated macrophages expressing MMP-12 below the basement membrane in the small bowel, thereby contributing to epithelial cell loss (53).

The Effects of MMPs in Stromal/Epithelial Interactions in Cancer

Tumor metastasis is a multi-step process in which a subset of cancer cells spread from a primary tumor to distant tissues. Tumor cells fulfill their metastatic potential after acquiring advantageous characteristics, allowing them to escape from the primary site, to migrate and invade the surrounding stroma, enter the vasculature, and establish metastatic foci (20). In the context of this chapter, we will focus on epithelial tumors of the gut and their relationship with stromal cells. It has now become apparent that MMPs in the tumor/stromal region appear to be more abundantly produced by stromal cells than tumor cells themselves. MMPs are not essential for tumor invasion, since tumor cells can use amoeboid movements to move into tissues (66). MMPs in human cancers, however, do more than just degrade ECM proteins and process a large number of growth factors, cytokines, chemokines, and other precursors into active moieties. The contribution of stromal cells to the expression of MMPs does not follow any particular pattern and appears to vary between the different types of tumors.

Animal studies on angiogenesis and metastasis

The complex role played by MMPs derived from the stroma in contributing to a suppressive effect on angiogenesis and a promoting effect on metastasis comes from studies in mouse models. Integrin alpha-1-deficient mice overexpress MMP-9 as well as the angiogenesis inhibitor angiotatin that is generated by the cleavage of plasminogen by MMP-9. Increased MMP-9 expression in this model has a paradoxical effect on tumor growth in that these mice exhibit a reduced number of growth foci in the primary tumor due to the inhibitory effects on angiogenesis but have an increased number of metastatic nodules in the lungs (12). Absence of MMP-2 or MMP-10 expression in knockout mice retards the formation of pancreatic islet tumors and skin tumors in transgenic models (8), implying a contributory role in carcinogenesis.

Experiments in transgenic mice have also revealed that stromal-derived MMPs contribute to metastasis (25). A decrease in the number of metastatic nodules in MMP-9-deficient mice and an increase in nodules in MMP-7-deficient mice following intravenous injection of lung carcinoma cells was recently reported (1). MMP-9 therefore appears to play a crucial role in the early survival and establishment of tumors rather than subsequent growth, which may be the reason for the failure of MMP inhibitors in clinical trials in patients with late-stage lung cancer.

Epithelial-mesenchymal transformation

Epithelial-mesenchymal transformation (EMT) is a critical step in the malignant transformation of epithelial cells into carcinomas. A common feature of EMT is the loss of the cell to cell adhesion molecule E-cadherin. MMPs such as MMP-3, MMP-7, and MT1-MMP have been shown to cleave E-cadherin, producing a peptide that stimulates cellular motility (44). MMPs have also been shown to be transcriptionally upregulated by β-catenin LEF/TCF complexes, which activate Wnt target genes as part of dysregulated Wnt signaling, suggesting a positive feedback mechanism whereby E-cadherin degradation by MMPs results in an increase in MMP expression (60). It is possible that overexpression of stromal-derived MMPs by carcinogens known to upregulate several MMPs could propagate epithelial transformation via this mechanism.

The contribution of MMPs to neovascularisation

Stromal-derived MMPs contribute to the formation of epithelial tumor vasculature by the solubilization of angiogenic factors such as vascular endothelial growth factor (VEGF). Solubilization of VEGF is required to initiate angiogenesis for the formation of pancreatic and skin tumors in transgenic mice (8). Mice deficient in MMPs have been shown to develop smaller tumors that grow at a slower rate and exhibit increased apoptosis and decreased density of vessels (36). Vasculogenesis is the recruitment of endothelial precursor cells (EPC) from the blood to become part of the tumor vasculature (48). Chemokines secreted by the tumor such as stromal-derived factor-1 (SDF-1/CXCL12) recruit EPCs to the primary site (51), with 2–5% of these cells contributing to the tumor vasculature. MMP-9 expression is upregulated by SDF-1, which causes shedding of the membrane associated mKitL (Kit ligand), which further enhances the recruitment of hematopoietic precursor cells and EPCs (21).

The induction of MMPs by malignant cells

Malignant cells express extracellular matrix metalloproteinase inducer (EMMPRIN/CD147) on their surface, which acts as a potent stimulator for the production of MMPs by stromal fibroblasts and endothelial cells (69). MMPs can also solubilize EMMPRIN, eliminating the need for cell contact for its effect (61). EMMP-
PRIN also stimulates the expression of MMP-1, -2, and -3 by endothelial cells and MT1-MMP by fibroblasts.

Tumor cells produce several growth factors and cytokines that also potently induce the expression of MMPs by stromal cells. For example, VEGF induces the expression of several MMPs in endothelial cells. Interleukin-6 (IL-6) and its soluble agonist receptor stimulate the expression of MMP-1 and -2 in bone marrow mesenchymal cells (5). MMP-9 expression in fibroblasts are also induced by tumor cell-derived TNF-α and TGF-β. This effect is dependent on smad-, ras-, and PI3K signaling, modulated by hepatocyte growth factor and EGF-mediated signaling (5).

The expression of MMP-7 is predominantly epithelial, when compared with most other MMPs. It has recently been reported that MMP-7 stimulated the proliferation and migration of myofibroblasts through the liberation of IGF II acting as an autocrine growth factor via the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways (23, 37). This raises the intriguing possibility of MMP-7 acting as a regulator and mediator of myofibroblast cell function, whereby epithelial cells may regulate stromal cell number and function in cancer.

Recruitment of MMP-producing cells

Tumor cells have also been shown to recruit MMP-2 and -9, producing CD45 positive neutrophils and macrophages into the tumor tissue by the production of colony-stimulating factor-1 (CSF-1), which is an important regulator of the mononuclear phagocyte lineage. CSF-1 is expressed in >70% of breast carcinomas and is associated with a poor prognosis (54). CSF-1 promotes metastatic potential by regulating the infiltration and function of tumor-associated macrophages at the tumor site. These macrophages are an abundant source of stromal-derived MMPs, where they contribute to tumor progression (FIGURE 3) (32).

Colorectal cancer

Multiple MMPs are overexpressed in a number of malignant tumors, and the expression and activation of these enzymes increase with tumor aggressiveness and metastatic potential and are associated with a poor prognosis. It was originally thought that MMPs were primarily made by cancer cells to degrade the surrounding ECM and basement membrane to invade and metastasize (33). MMP-mediated proteolysis of ECM has also been shown to release biologically active molecules such as TGF-β that are anchored in the matrix and contribute to cell proliferation, invasion, and survival of the tumor cells (24). Human colorectal tumors have been shown to express MMP-1, -2, -3, -7, -9, -10, -11, -12, -13, and -14. Measuring these enzyme levels has been shown to help with prognosis. For example, patients with a high level of MMP-9 mRNA had six times more recurrent disease (63.4%) compared with patients with low levels of MMP-9 mRNA (70). In contrast to other MMPs, expression of MMP-12 is associated with approximately twofold increased survival (76%) compared with patients without MMP-12 expression. This improved survival may be a result of the inhibitory effect of MMP-12 on angiogenesis (71).

Concluding Remarks

Mesenchymal/stromal cell interactions in health and disease involve MMPs and involve multiple ligands and cellular signaling pathways. The variations in the levels of MMPs between the epithelium and the stroma, their cross talk, and associated interactions are likely to be critically important in the development of a number of gastrointestinal disorders such as inflammatory bowel disease and cancer.

References


