ROLE OF MATRIX METALLOPROTEINASES (MMPS) IN COLORECTAL CANCER (CRC): REGULATION AND THERAPEUTIC INTERVENTIONS

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Abstract : The matrix metalloproteinases (MMPs) comprises of proteases that are well regulated with their endogenous anti-proteinases in many physiological processes with the right expression and stoichiometry. The dysregulation of that metalloproteinases and anti-proteinases play important role in several pathological processes and more viciously in tumour progression and the metastasis. It has been shown by investigators that matrix metalloproteinases (MMPs) play important role in colorectal cancer (CRC) invasion and metastasis. The MMPs able to degrade and/or activate proteins, that play crucial role in tumour initiation, progression and metastases, suggesting that proper regulation and/or management of MMPs during colorectal cancer would be the possible diagnostic, prognostic and therapeutic targets of patients of CRC. Therefore, MMPs have been unanimously considered as the advanced and one of the therapeutic strategies focusing on mainly down regulating or blocking these proteinases by several natural as well as synthetic inhibitors. In fact, many clinical trials by researchers using MMP inhibitors were going on to manage this disease by so many different and efficient ways. Based on this evidence, the pharmaceutical industry produced several well tolerated, active MMP inhibitors (MMPIs) which demonstrated efficacy in different CRC models too.

Keywords: matrix metalloproteinases, tissue inhibitor of metalloproteinases, MMP inhibitors, tissue remodeling, inflammation, wound healing, colorectal cancer

Abbreviations: matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinases (TIMPs), MMP inhibitors (MMPIs), extracellular matrix (ECM), colorectal cancer (CRC)

1. INTRODUCTION

Cancers that affect either or both of colon and/or rectum organs may be called colorectal cancer. The majority of colorectal cancers generally develop over time from adenomatous (precancerous) polyps. Polyps (growths) can change after a series of mutations (abnormalities) arise in their cellular DNA. Some of the risk factors for colorectal cancer involve a family history of colon or rectal cancer, diet, alcohol intake, smoking and inflammatory bowel disease.

Colorectal cancer (CRC) is one of the leading causes of worldwide cancer mortality. Despite the significant advances in diagnosis, screening and treatment, there are limited therapeutic options for patients with advanced disease, highlighting the need for additional tumour molecular biomarkers and prognostic predictors [1-7]. Colorectal Cancer (CRC) pathogenesis results from a complex, multistage process progressing from neoplastic transformation of normal cells, tissue invasion, vascular intra- and extravasation, and ultimately seeding in another organ. In all tissues, the extracellular matrix (ECM) provides a structural and biochemical framework for cell support and scaffolding, with a range of functions important for regulating both inter- and intra-cellular signaling, and for cellular differentiation, adhesion and invasion. Cancer cells interact with the ECM, and structural remodeling is important for migration from a primary tumour site. Proteins comprising the ECM play critical roles in cell proliferation and migration, and different proteases control ECM remodeling and degradation. One

specific group of proteolytic enzymes, matrix metalloproteinases (MMPs), were studied extensively as key mediators of ECM degradation and in the processing of other bioactive molecules [8]. In this respect, not only Matrix Metalloproteinases (MMPs) and but also their tissue inhibitors (TIMPs) have been widely recognised playing a predominant role in cancer cells, especially in the processes of tumour invasion, progression and metastasis of CRC. In patients with CRC the roles and functions of MMPs, their diagnostic sensitivity as biomarkers and utility as pharmacological targets is a promising and a challenging area for investigations nowadays. Even though the prognostic value of some MMPs is a matter of literature debate, several studies and meta-analyses have revealed the association among over-expression of some MMPs with worse outcome, poorer overall progression-free survival, suggesting them as prognostic indicators and potential target for treatment in CRC patients (Table1) [8-10].

Matrix metalloproteinases have Zn^{2+} ions at their active site-pocket and they require an optimum concentration of Ca^{2+} ion for their maximum catalytic activities (Figure 1). The proteinases were discovered more than fifty years ago and since then have been identified as the more responsible macromolecules for extracellular matrix (ECM) homeostatis [11-15]. They comprises of a large number of metalloproteinases in their superfamily including the most classical MMPs. Beside this, they are simply categorized according to their expression and location in human like membrane-bound MMPs (MT-MMPs), adamlysins (ADAMs) and a disintegrin and metalloproteinase with thrombospondin motif (ADAMTS) [16-18]. Their disticnct nomenclature was done mostly according to their substrate specificity like collagenases, gelatinases, stromelysins, elastases and aggrecanases (Table-2).

MMP activity can be controlled at different levels: transcription, proteolytic activation of the zymogen form, and inhibition of the active form [19-21]. A group of endogenously secreted proteins called tissue inhibitor of metalloproteinases or TIMPs are the determinant for the *in vivo* or *in vitro* activities of MMPs (Table-3). Tissue inhibitors of metalloproteinases (TIMPs) are extracellular matrix proteins which are recognized as most appropriate and physiological endogenous regulators or inhibitors of MMPs (Table-3). To date, five have been identified, designated as TIMP-1 to 5, as small molecules of 20-30 kDa that reversibly bind and block MMPs [22-25]. The TIMPs are secreted proteins which complex with individual MMPs and have a central role in regulating both the functional activity and activation of individual MMPs [25-28]. It is thought that the balance between activated MMPs and TIMPs determines overall MMP activity and proteolysis *in vivo*. TIMPs differ in their specificity towards MMPs and in their expression pattern [22-28].

Now the understanding of the regulation of MMPs and TIMPs is notably studied so far. Many studies have been developed practically not only to study MMP structure/function but also their relationships with MMP inhibitors in tuning up of numerous normal and disease processes [16-20]. In addition, development of MMP null mice has given us an excellent direction of fruitful research to understand exactly the role of MMPs not only in physiological processes but also in diseases including cancers [21-27]. If limited proteolysis of MMPs is not controlled precisely then dysregulation can cause to several pathological conditions. After all this dysregulation develop uncountable diseases processes like inflammation, cardiovascular diseases such as stroke, periodontitis, ulceration, arthritis, fibrosis, emphysema, diabetes complications, disorder in nervous system such as parkinson's disease, alzheimer's diseases and cancer [29-30].

Molecular pathways critical to the regulation of MMP gene transcription as well as MMP synthesis and activation of pro-MMPs during normal development, directed to the researchers in the universe to confine to discover the endogenous inhibitors of MMPs and afterwards the development of synthetic MMP inhibitors [31, 32] that could be employed to study their effectiveness in regulating abnormal ECM turnover in animal models of disease. Intracellular signaling pathways involving stress-activated protein kinases and tyrosine-receptor protein kinases that regulate MMP gene expression after

cytokine, chemokine or growth factor activation have also been uncovered [33]. It can now be hypothesized that experimental manipulation of intracellular signaling pathways may be feasible for devising novel therapeutic strategies for treating numerous life threatening diseases. Recently, several investigators are engaged to produce very specific inhibitors to manage selected MMPs in multiple ways that are responsible for different diseases more significantly various type of cancers [30-35].

Tumor invasion and metastasis is a highly complicated, multi-step phenomenon. In the complex event of tumor progression, tumor cells interact with basement membrane and extracellular matrix components. Proteolytic enzymes (proteinases) are involved in the degradation of extracellular matrix, but also in cancer invasion and metastasis. Different investigators have shown that proteolytic enzymes play a major role not only in colorectal cancer (CRC) invasion and metastasis, but also in malignant transformation of precancerous lesions into cancer. Tissue and serum plasma antigen concentrations of proteinases might be of great value in identifying patients with poor prognosis in CRC. It has been indicated that the potential tumor marker impact of proteinases for the early diagnosis of CRC. In addition, proteinases may also serve as potential target molecules for therapeutic agents [36-47].

Nowadays it is clear to us that abnormal balance between the MMPs and TIMPs is considered a key factor in the development of various diseases. This dysregulation of the stoichimetry of MMPs/TIMPs mostly responsible for the elevated level of MMP activity which furher help people to find out of inhibition or regulation mechanism of MMPs to discover an effective therapeutic strategy in different diseases. In spite of several positive results to manage MMPs inhibition or regulation, but there are so many deficiencies in these studies like the lack of selective findings of natural and synthetic MMP inhibitors, lack of knowledge regarding the exact functions and interactions of a particular MMP towards TIMPs, fails to produce novel therapeutics in clinical applications are to be considered the serious problems during the studies [26-30].

The inability to control metastasis is the leading cause of death in patients with cancer. Control of metastasis, therefore, represents an important therapeutic target. Based on a sound understanding of the biochemistry of matrix metalloproteinases (MMPs) and the accumulation of considerable experimental evidence implicating MMPs in cancer dissemination, the pharmaceutical industry has invested heavily in developing effective MMP inhibitors (MMPIs) for the treatment of cancer. Since MMPs also play an important role in tumour angiogenesis, MMPIs may have a dual role in the treatment of cancer [30-35].

In the other hand, the failure of MMPIs to alter disease progression in metastatic cancer might have been anticipated since MMPs appear to be important in early aspects of cancer progression and may no longer be required once metastases have been established. In spite of considerable recent progress in identifying multiple roles of MMPs in disease, the understanding of MMP function in cancer is far from complete. Based on accumulated data, it is recommended that future MMPI trials focus on: (1) patients with early stage cancer; (2) the use of MMPIs along with chemotherapy; (3) the measurement of MMPs in tumour tissue and blood as a means of identifying patients who are more likely to respond to MMPI therapy; and (4) identification of biomarkers that reflect activation or inhibition of MMPs [30-40].

2. EVENTS DURING TUMOR INVASION AND METASTASIS

Tumor invasion and metastasis is a multi-step event. It has been shown that cancer metastasis is a complex series of sequential processes: the initial transforming event; proliferation of transformed cells; the ability of tumor cells to avoid destruction by immune-response; nutrition supply to the tumor mass; local invasion and destruction of extracellular matrix components (ECM); migration of tumor cells; penetration of tumor cells through the blood vessel wall; embolization of tumor cells and adhesion to distant organs; arrest of cancer cells in the lumen of small blood vessels and lymphatics; reverse penetration of blood vessels and formation of distant metastases [48–52]. In the complex event of tumor

invasion and metastasis, tumor cells are tightly interacted with basement membrane (BM) and ECM. Three steps have been suggested to describe the sequence of events during tumor cell invasion of ECM: attachment, matrix dissolution and migration. The first step is tumor cell attachment to the matrix. The attachment is mediated by tumor cell surface receptors, when tumor cells bind to the BM surface. This process is mediated by specific glycoproteins such as fibronectin and laminin. During the second step (local degradation of matrix by tumor cell-associated proteinases) tumor cells directly secrete enzymes to degrade ECM. Such proteinases can degrade both the structural collagenous proteins of the matrix and the attachment proteins. During the third step (migration), cancer cells are migrating across the BM and stroma through the zone of matrix proteolysis. Invasion of ECM is accomplished by cyclic reverberation of these three steps. Chemotactic factors can influence the direction of migration [53-60].

3. MATRIX METALLOPROTEINASES AND CANCER

MMPs play a salient role in all type of cancer known so far [61-65]. MMPs play salient role in tumor development and growth as well as metastasis [66-69]. MMP-2 and MMP-9 (72kDa gelatinase and 92kDa gelatinase) are the prominent MMPs responsible for basement membrane ECM protein degradation that facilitates the migration of tumor cells to blood vessels. In one aspect unrelated to the capacity of MMPs to degrade ECM proteins, MMPs are intimately involved in stimulating angiogenesis which is required for tumor progression [70-75]. In conjunction with its ability to stimulate neovessel formation, MMP-9 also appears to be active in releasing tissue-bound fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) that facilitates tumor cell growth. In another aspect, single nucleotide polymorphisms that result in elevated MMP gene expression also appear to be associated with the DNA from patients with more advanced cancer, suggesting that elevated MMP levels contribute to cancer progression [76]. MMPs may also be involved in dysfunctional apoptosis [77] and altered immune-mediated tumor killing [78] that are both characteristic of malignancy. In this regard, specifically designed synthetic MMP inhibitors are not likely to prove efficacious as a cancer therapy if they interfere with anti-angiogenesis pathways or immune-mediated tumor killing.

Tumor cells produce proteolytic enzymes that can destroy the matrix barriers ambient the tumor, permitting invasion into surrounding connective tissues. MMPs are able to degrade virtually all components of the ECM and connective tissue surrounding the tumor cells and the basement membrane. It was initially believed that MMPs are being produced and secreted by tumor cells, degrading basement membrane and ECM components. Now, we learned that MMPs are also produced by surrounding stromal cells, including fibroblasts and infiltrating inflammatory cells. It was initially believed that MMPs, via breakdown of the physical barrier, were primarily involved in tumor invasion. There is growing evidence, however, that MMPs have an expanded role, as they are important for the creation and maintenance of a microenvironment that facilitates growth and angiogenesis of tumors at primary and metastatic sites. In cancer, MMPs are involved in angiogenesis by regulating the bioavailability of vascular endothelial growth factor (VEGF) (e.g., MMP-9) and the cleavage of matrix-bound VEGF (MMPs-3, -7, -9). Further, MMPs can influence the balance between growth signals and growth-inhibiting signals (by activation of the epidermal growth factor (EGF) receptor and modulation of the transforming growth factor-β (TGF-β) pathway), regulate the induction of apoptosis by cleavage of Fas ligand (MMP-7), control inflammation (MMP-2, -3, -7, -8, -9, -12), play a part in the creation of metastatic niche (MMP-3, -9, -10) and invasive processes (MMP-1, -2, -7, -9, -13, -14) [34–41].

Different studies demonstrated that MMPs, among them particularly type IV collagenase MMP-9 (gelatinase B), are especially important in the process of tumor invasion and metastasis, but also in the remodeling and inflammatory processes in Inflammatory bowel disease (IBD) [42–50]. Several MMPs are expressed by tumor cells in oral squamous cell carcinoma [51,52], prostate cancer [53], breast cancer

[54], ovarian cancer [55–57] and in many GI tumors such as esophageal squamous cell and adenocarcinomas [58–60], gastric cancer [61,62], pancreatic cancer [63,64] or hepatocellular carcinoma [65,66]. In the specific case of MMP-9, some researchers investigated the role of MMP-9 expression using immunohistochemical analysis in the development and progression of reflux esophagitis- Barrett's esophagus-dysplasia-adenocarcinoma sequence in the esophagus. Increased immunohistochemical expression of MMP-9 in Barrett's metaplasia-dysplasia-adenocarcinoma sequence as compared to normal tissue suggested its association with esophageal tumorigenesis. Increased expression of MMP-9 in Barrett with dysplasia compared to non-dysplastic metaplasia indicated that this alteration might be early event in carcinogenesis. They suggested that quantification of MMP-9 in Barrett's esophagus might be useful to identify patients at higher risk for progression to esophageal adenocarcinoma [42]. In an immunohistochemical study they demonstrated that the mucosal up-regulation of MMP-9 correlated with the severity of inflammation in ulcerative colitis (UC), suggesting that the increased MMP-9 expression could contribute to the severity of mucosal damage in active UC. MMPs have been also considered as potential diagnostic and prognostic biomarkers in many types and stages of cancer [67,68].

4. TISSUE INHIBITOR OF METALLOPROTEINASES (TIMPS) AND CANCER

MMPs are controlled by endogenous tissue specific inhibitors called tissue inhibitors of metalloproteinases (TIMPs), which are secreted proteins. TIMPs bind and inhibit enzymatically active MMPs at a 1:1 molar stochiometric proportion thus inhibiting the proteolytic activity of MMPs. The impact of TIMPs is essential for the homeostasis of the ECM. The sensitive balance between MMPs and TIMPs is essential for many physiological processes in the gut [69–74].They have recently demonstrated that not only MMPs but also TIMPs may contribute to the inflammatory and remodeling processes in IBD and serum TIMP-1 might be useful as additional biomarker in the assessment of IBD activity [50]. The imbalance between MMPs and TIMPs is an essential step in the development of GI malignancies and is of critical importance in early events of tumor progression. TIMPs might display a complex and dual influence on tumor progression and metastasis: on one hand they directly regulate and inhibit MMPs, on the other hand influence angiogenesis, inhibit the apoptosis of tumor cells, malignant transformation and facilitate tumor growth and metastasis [10,44,75–79].

Regulation of cell to cell and cell to matrix adhesion is controlled in normal cells, while disturbance in ell adhesion is common in human tumors. The relationship among cancer cells with the ECM and adjacent cells is accomplished through ECM components, adhesion molecules, proteinases and their endogenous tissue specific inhibitors. MMPs degrade the ECM and prepare the route for cancer cells to migrate, invade and spread to distant zone, forming metastasis. Active MMPs target ECM ligands for degradation (including laminin, collagens, vitronectin, fibronectin), resulting in ECM cancer cell detachment and remodeling. Active MMPs target also tumor cell adhesion receptors, such as integrins or E-cadherin and disrupt both cell- to cell and cell- to matrix adhesions. Tumor cell specific mechanisms, such as tumor angiogenesis or epithelial to mesenchimal transition (EMT) leads to increased MMPs activity. Ultimately, reduced MMPs inhibitory mechanisms may disrupt the MMPs/TIMPs ratio within the tumor microenvironment facilitating distant metastases [78–82]. Four TIMPs have been characterized in humans (TIMP-1, -2, -3 and TIMP-4), which share notable homology and structural identify at the protein level. TIMP-1 inhibits angiogenesis either directly by an as yet unknown mechanism or indirectly thorough restraining MMP-9-mediated release of vascular endothelial growth factor (VEGF) from matrix. TIMP-2 selectively block human microvascular endothelial cell growth in vitro in response to proangiogenic factors such as fibroblast growth factor-2 (FGF-2) or vascular endothelial growth factor A (VEGF-A). TIMP-2 could also suppress receptor tyrosine kinase signaling independent of MMP inhibition. TIMP-3 has been demonstrated to promote apoptosis in several in vitro systems. TIMP-3 has

been also identified as a tumor suppressor for adherent malignant and normal cells. TIMP-3 inhibits adhesion of cells to ECM and promotes apoptosis through death-receptor-activated, caspase-8-mediated pathway. TIMP-4 enhances or inhibits the in vivo growth of tumor xenografts. It has also been shown that although TIMP-4 inhibits capillary endothelial cell migration in vitro it does not inhibit angiogenesis induced by FGF-2 in experimental systems [75,78,80–82].

Pathological angiogenesis is a hallmark of cancer. Angiogenesis is a complex process regulated by growth factors and by the force balance between endothelial cells traction stresses and ECM viscoelastic resistance. In vitro studies suggest that decreasing ECM stiffness can trigger an angiogenic switch. It has been demonstrated that MMPs should play an important role in this switching mechanism. Uncontrolled MMP activity results in tissue damage and functional alterations. MMP/TIMP physiological equilibrium is shifted in malignant tissues. During tumor progression there is an increase in secretion and activation of MMPs by either the tumor cells themselves or tumor-associated fibroblasts, launching the formation of tumor microenvironment. The increase of MMPs activities is initially inhibited by TIMPs. During tumor growth, more and more MMPs are secreted, overgrowing the local TIMPs secretion. This imbalance between MMPs and TIMPs can contribute to the ECM remodeling. Moreover, further tumor progression and growth drives local tissue hypoxia and MMP-mediated release of angiogenic factors. These steps facilitate the tumor-related angiogenic reaction, which also require MMP activity [78,79, 83–86].

5. THE ROLE OF MMPS AND TIMPS IN COLORECTAL CANCER

Several studies have demonstrated that the expression of several MMPs and TIMPs are enhanced in CRC (Table-1). CRC is characterized by enhanced expression of several MMPs, such as MMP-1, MMP-2, MMP-7 or MMP-13, but one particular MMP, the type IV collagenase, MMP-9 (gelatinase B) is of special interest with respect to the development and progression of CRC. Previously some preliminary studies suggested that MMP-9 expression was related to prognosis [87–90]. More recently, in an immunohistochemical study they have demonstrated that tissue expression of MMP-9 was significantly higher in moderately (G2) and poorly (G3) differentiated tumors than in well differentiated (G1) cancers, as well as in advanced Dukes stages compared with Dukes stage A. They have shown diffuse strong MMP-9 expression in both tumor and stromal cells. For confirmation of the immunohistochemical results MMP-9 TaqMan real-time RT-PCR analysis was performed by various groups. The RT-PCR results (using whole tissue lysates) correlate with the immunohistochemical behavior of MMP-9 in the colonic mucosa, showing a significantly higher expression of MMP-9 in cancer tissue compared to normal colonic mucosa [43]. Furthermore, recent immunohistochemical studies have confirmed that tissue MMP-9 can serve as an independent prognostic marker in CRC and quantification of MMP-9 expression may be valuable in finding patients who are at high risk of developing disease recurrence [91,92]. Recent study demonstrated MMP-9 immunohistochemical expression was significantly positively correlated with depth of CRC invasion, lymph node metastasis and distant metastasis [93]. Moreover, multivariate analysis adjusted for age, gender, tumor location, differentiation status and stage proved that MMP-9 expression was an independent marker of worse disease-free and overall survival, concluding that MMP-9 could serve as a novel prognostic marker that is additive to the tumor-node-metastasis (TNM) staging system. Very recent study showed that high protein expression of MMP-9 and MMP-2 in normal mucosa is also associated with worse five-year survival, indicating that increased MMP-9 and MMP-2 protein expression in normal mucosa of CRC patients is prognostic for survival [94]. In addition, it has also been demonstrated that not only MMP-9 and MMP-2, but also tissue expressions of MMP-1 and TIMPs might be useful prognostic markers and predictors of liver metastasis [95–97]. Moreover, very recently it has

ben demonstrated that expressions of MMP-11 by fibroblasts and MMP-13 by tumor cells were also associated with poor prognosis [98]. It has been postulated that colonic and rectal carcinomas may have different mechanisms of carcinogenesis. Further, patients with rectal cancer are considered to have a poorer survival than those who suffer from colon cancer [99,100]. Recently it has been reported that in a specific case of rectal cancer tissue expression of gelatinases (MMP-2 and-9) also had a possible prognostic significance [101,102].

It has been proposed that MMPs and TIMPs might play a part not only in tumor invasion and initiation of metastasis but also in CRC carcinogenesis from colorectal adenomas. The adenomatous polyps are neoplastic tumors with a potential to progress into invasive CRC. The expression of MMP-9 and TIMP-1 in the normal mucosa-adenoma-dysplasia-adenocarcinoma sequence of the colon was studied by few authors. It has also been demonstrated that immunostaining of MMP-2 and MMP-9, as well as TIMP-1 and TIMP-2 increased gradually from tubular to villous adenomas, while in situ carcinomas showed a definite positive expression, concluding that the behavior of MMP-2, MMP-9 and TIMPs coincides with a multistep process of colonic tumorigenesis [103]. It has also been shown that protein expression of MMP-9 in CRC was significantly higher compared to adenomas and the normal mucosa. In addition, they demonstrated higher tissue expression of MMP-9 in adenomas with HGD compared to other adenomas or normal colonic mucosa. In adenoma samples, dysplastic epithelial cells showed moderate intensive cytoplasmic MMP-9 expression, with a clear-cut differentiation between dysplastic and non-dysplastic areas, indicating that the overexpression of MMP-9 may be an early event in colorectal carcinogenesis [43].

6. DIAGNOSTIC VALUE OF MMPS AND TIMPS

The potential tumor marker impact of MMP-s and TIMPs has been extensively studied. It was clearly shown by several authors that MMP-9 and TIMP-1 have significant potential as biomarkers in CRC. Diagnostic sensitivity of MMP-9 and TIMP-1 was consistently higher compared with those of conventional biomarkers Cancer embryonic antigen (CEA) and carbohydrate antigen (CA); (CEA or CA 19–9). Is has been suggested that MMP-9 and TIMP-1 estimation likely have the greatest predictive impact when screened as part of a biomarker panel [118–120]. In a very recent study, [121] evaluated the accuracy of MMP-9 for CRC in an asymptomatic population. From 748 patients overall, 46 cases of CRC were identified. Univariate analysis showed that increased serum MMP-9 concentration, demographic characteristics and behavioral factors were all significantly associated with presence of CRC.

A multivariate analysis showed that TIMP-1 and CEA were significant and independent markers of the presence of both colon and rectal cancers. This prospective validation study suggests the use of the combination of plasma TIMP-1 and CEA protein determinations as an additional support in early detection of CRC [122].

Although the fecal occult blood test (FOBT)-based colorectal screening is likely reducing the incidence and mortality of CRC, the test gives high false positive as well as negative results, therefore there is a need to improve the screening test, ideally to increase the positive predictive value. In a pilot study of 300 patients attending the Queen Elizabeth Hospital, high serum MMP-9 levels accurately predicted in CRC patients. The results of this pilot study suggest that MMP-9 may be an effective secondary screening test [123, 124].

7. PROGNOSTIC VALUE OF MMPS AND TIMPS

In a preliminary study some people have demonstrated that serum antigen concentrations of MMP-2, MMP-7, MMP-9 as well as TIMP-1 and TIMP-2 were significantly higher in patients with CRC than in healthy controls. In addition, all examined parameters were significantly higher in patients with

CRC than in patients with adenomas. Higher serum antigen concentrations of MMPs and TIMPs significantly correlated with tumor stage, nodal involvement and the presence of distant metastases. The results obtained by different researchers: from blood samples confirm previous results of tissue expressions concluding that MMPs and TIMPs play an important role in CRC invasion and metastasis, and they are also activated in premalignant colorectal adenomas. The increasing serum antigen concentrations of MMP-s and TIMPs coincide with a multistep process of colonic carcinogenesis. Furthermore, they suggested that measurement of MMPs and TIMPs might be useful in the assessment of preoperative tumor stage [125]. It has been revealed by the studies that serum concentrations of MMP-9 and TIMP-1 were significantly higher in adenoma patients compared with control group but lower than in patients with CRC. Several studies confirmed that high preoperative serum or plasma MMP-2, MMP-9 and mainly TIMP-1 antigen levels are strong prognostic factors for patients with CRC and their determination might be useful for identification of patients with higher risk for cancer recurrence. Preoperative blood-levels of TIMP-1 were independent predictors of disease-free survival in patients with primary resectable CRC [118,119,126,127]. Very recently, [128] a high serum levels of TIMP-1 were correlated with CRC liver metastasis and were significant predictive factors for poor prognosis following resection of synchronous liver metastasis.

In a pilot study, it has been measured MMPs in postoperative intraperitoneal fluid after rectal cancer surgery. They found that elevated MMP-8 and MMP-9 levels were markers for later development of anastomotic leakage after surgery. They suggest that MMPs appear to have an important role in the development of anastomotic leakage and may be promising pharmacological targets to protect anastomotic integrity [129].

The immunoassay kits of MMPs and TIMPs are usually designed for determination of concentrations in cell culture supernates, serum and/or plasma. For quantitative comparison in humans, plasma and/or serum concentrations are accepted to use, however the use of serum MMPs and TIMPs have been previously criticized due to its increased level compared with plasma estimation [130,131]. It is well known that MMPs are stored in macrophage and neutrophil granules, while most of TIMPs are secreted by platelets. Therefore, when using serum levels of MMPs and TIMPs, there are 3- to 5 fold higher levels than in corresponding EDTA or citrate plasma samples. Despite citrate-plasma being the suggested sample of choice for estimating circulating MMPs or TIMPS [132], serum sampling may still be useful provided that methods of collection and processing were standardized [121-131]. Thus, one should be aware of the pre-analytical pitfalls to avoid misinterpretation of data when determining MMP and/or TIMP levels. Further, when collecting samples it is recommended after centrifugation to aliquot and store samples at −20 °C or −80 °C or assay immediately. The time elapsed between blood sampling and centrifugation is associated with higher serum MMPs levels, with a suggested seven-fold increase after 2 h [130-133]. MMPs degrades during storage, even at −80 °C, therefore the repeated freeze-thaw cycles should be avoided, while TIMPs are stable and can be frozen/thawed for several times.

8. PREDICTIVE VALUE OF MMPS AND TIMPS IN RESPONSE TO CHEMOTHERAPY

It has also been suggested that TIMPs can predict individual responses to chemotherapy. In the study of some investigators [134] patients with metastatic CRC were included. Plasma TIMP-1 and serum CEA were measured in samples obtained before the first cycle of first-line combination chemotherapy. It was shown that plasma TIMP-1 concentrations obtained before the first cycle of chemotherapy were significantly and independently associated with objective response, time to progression (TTP) and overall survival (OS) of patients with metastatic CRC receiving combination of irinotecan, 5-fluoruracil (5-FU), and folinic acid chemotherapy. CEA was not significantly associated with TTP or OS when TIMP-1 was included in the multivariable analysis. One explanation for these associations is that TIMP-1 protects

cancer cells against the apoptotic stimuli that consecutively affect the cells. It has been demonstrated previously that TIMP-1 possesses antiapoptotic effects, which might be enhanced by administered chemotherapy. The antiapoptotic effect of TIMP-1 can be regulated in a MMP-dependent, and a MMP-independent way as a consequence of both direct effects on tumor cells and modulation of the tumor microenvironment [135]. In vitro and in vivo studies have shown that apoptotic effector molecules, such as caspases, are induced by degradation of ECM by MMPs, leading to apoptotic cell death. The anti-MMP function of TIMP-1 would indirectly inhibit apoptosis [136–139]. Knowing that TIMP-1 can induce chemoresistance in cancer cells in vitro, one can speculate whether TIMP-1 could be a real target for increasing tumor cell sensitivity to chemotherapy. Further prospective studies are needed to validate plasma TIMP-1 measurements in the prediction of response to chemotherapy.

Another study evaluated the effect of chemotherapy on plasma TIMP-1 antigen concentrations in comparison with CEA levels in patients with stage III CRC. Thirty patients with primary CRC, who had been intended curatively resected for stage III disease and scheduled for adjuvant chemotherapy, were prospectively included before the initiation of chemotherapy. Patients received 10–12 cycles of chemotherapy of a modified FOLFOX 6 regimen. Plasma CEA levels were stable during the first and second cycle of chemotherapy, while the plasma levels of TIMP-1 were directly affected by chemotherapy represented by a significant, but transient increase after two weeks following the second treatment and a recovery to normal three months later. According to this, TIMP-1 can be considered as an additional tool for monitoring chemotherapy in CRC [140]. The mechanism behind the observed increase in plasma TIMP-1 may be partly associated to cellular disintegration (tumor cells and/or blood cells) with subsequent release of soluble TIMP-1. The disintegration of platelets induced by chemotherapy and in particular to oxaliplatin is well known and might contribute to the raise of plasma TIMP-1 levels. Another explanation could be the up-regulation of TIMP-1 synthesis and release as a response to apoptosis induced by the chemotherapy [140–144].

It has been [145, 146] demonstrated an essential role of TIMP-1 in the anti-invasive action of cisplatin on human cancer cells. It is suggested that this mechanism can play an important role in the antineoplastic actions of this prominent chemotherapeutic agent. In another study, the same group demonstrated that in human cancer cell lines increased expression of TIMP-1 mediates an anti-invasive effect of cannabinoids. It is well known, that cannabinoids, in addition to having palliative benefits in cancer therapy, have also been associated with anticarcinogenic effects [147]. Consistent with this finding, the anti-invasive action of several anticarcinogenic substances has been associated with elevated TIMP-1 levels [47–49]. Watanabe et al. [147] in a patient population comprising 25 patients with metastatic CRC treated with bevacizumab with either modified FOLFOX 6 or FOLFIRI, from whom tumor samples were available for gene expression analysis, showed that a model of genes for VEGF-A, thymidylate synthase and TIMP-3 predicted clinical response to bevacizumab therapy with an accuracy of 96%, sensitivity of 91%, specificity of 100%, and positive and negative predictive values of 100% and 93%, respectively, suggesting that the above predictive model may be useful in selection of CRC patients who would benefit from bevacizumab treatment.

9. PHARMACOLOGICAL TARGETING OF MMPS

Several therapeutic MMP inhibitors (MMPIs) have been developed to target MMPs, attempting to control their synthesis, secretion, activation and proteolytic activity. Several generations of synthetic MMPIs were under investigation in phase III clinical trials in recent years. Although there have been important preclinical and clinical studies on the agents targeting MMPs, most of these agents failed in clinical trials due to inefficacy and adverse side effect, thus they are yet not available for routine use as therapeutic agents [51–56]. The failure of MMPIs can be attributed to the following: (a) poorly defined

predictive preclinical animal models for safety and efficacy; (b) limited knowledge of the variety of biological functions of MMPs; (c) poor selectivity of the MMPIs: due to homology between catalytic domains of MMPs none of the agents were highly selective for specific MMPs; (d) poor target validation for the targeted therapy: entry criteria excluded patients with early stage cancer, while MMPIs appear to be moreactive in early, rather than in late cancer stage (e) unexpected long-term drug intolerance reduced treatment compliance; (f) drug-dosage on short-term studies in healthy volunteers were not predictive of long-term therapeutic drug concentrations reached in cancer patients; (g) some MMPs exhibit antitumor activity; (h) MMPs are mainly produced by stromal cells, rather than the tumor cells themselves, therefore the exact cellular target of MMPIs was not precisely defined [57–59]. The most frequent severe adverse side effect associated with the clinical trials of MMPs was a musculoskeletal syndrome (MSS) that manifested as immobility and pain in the shoulder joints, arthralgias, contracture in the hands, and an overall impaired quality of life for patients. It was shown that development of MSS was time and dose-related. It has been suggested that development of MSS was the best indicator of dose optimization and successful MMP inhibition [60–62]. On the other hand, it should be always kept in mind that MMPs and TIMPs participate in regulation of tissue remodeling in healthy persons and in normal, non-cancerous tissue even in cancer patients. As such inhibition or blockade of these proteins will have influence on normal functions that may take place even in cancer patients. In addition, most CRC patients may also have concomitant disorders such as cardiovascular, hepatic or endocrine disorders, which also might be influenced by treatment with MMPIs [53,63,64].

The failure of MMPIs as cancer drugs in clinical practice suggests that the understanding of the molecular and cellular events involved in tissue remodeling is incomplete. In the light of the knowledge the proteolytic enzyme inhibitor approach seems no longer be sufficient because it does not affect the interactions of MMPs with cell surface proteins and consequents signaling [65,78]. The development of a new generation of selective inhibitors or monoclonal antibodies highly specific for certain MMPs is a promising area of research. New therapeutic strategies are focusing on more selective MMPIs: newly suggested inhibitors include peptides that block exosite-mediated cell surface interplay and/or function-blocking anti-MMP antibodies [78,66-71]. Furthermore, taken into consideration the high molecular complexity of tumor progression, combination of MMPIs with conventional chemotherapeutic or molecular targeted agents may also increase the effectiveness of oncological therapy [53,72].

10. PERSPECTIVE AND FUTURE DIRECTIONS

The last decade has seen a remarkable increase in the number and quality of publications on the importance of MMPs both in physiological and pathological states. All are concerning the mechanisms of cell/tissue-specific transcriptional regulation of MMP gene expression and the attendant signal transduction pathways holds hope for the rational design of pharmaceuticals that control the production of MMPs in a targeted fashion. MMPs occupy a secure position as major determinants for normal embryonic development as well as for tissue injury which are characteristic of progression of various inflammatory disease processes. Assessing the relevancy of specific MMP inhibitors for use in regulating aberrant ECM protein turnover should occur first in animal models of skeletal dysplasias, cardiovascular abnormalities, arthritis, cancer and CNS disturbances as the results of these studies will likely be of the utmost importance for judging the potential usefulness of these MMP inhibitors in human clinical trials. The design of MMP inhibitors for use in the clinic must also take into account their potential for disrupting critical pathways required for tissue homeostasis.

Now a large number of MMP inhibitors have been designed and synthesized or else harvested from natural sources which are clinically important so far [104-110]. But there are some failures during the treatment of diseases that have been observed yet to be resolved as far as the efficacy of these

inhibitors concerned due to the limited knowledge of the function of all TIMPs towards each of the MMPs in biology and pathology and undoubtedly the lack of selective inhibitors. Now we are in a very crucial and challengingsituation to design the selective inhibitor(s) by identifying thevery specific MMPs, or set of MMPs, or other enzymes and nonenzymes which are exactly responsible for the progression different critical diseases prevalent to human. All type of studies will provide to screen out and to designof novel types of allosteric inhibitors of MMPs for therapeutic intervention.

Although it is now clear those TIMPs are multifunctional those have MMP-inhibitory activities and also MMP regulatory effect and their expression and function should be exploited in the search for novel therapies. This is done either inhibition of MMP activity by small molecule or drugs or increasing the local concentration of TIMPs by recombinant protein administration or gene transfer. The uses of synthetic MMP inhibitors (MMPI) as clinical trial raise so many disappointing and doubtful results mainly due to their lack of efficacy and untoward side effects rather than the natural inhibitors.But the studies of synthetic inhibitors is praisworthy like marimastat, illomastat, rebimastat, tranostat batimastat, Ro28-2653etc for the treatment of diseases especially in cancer progression and survival. This suggests that, we have to know more about the precise roles of MMPs in different disease proceeses to implicate its clinical manifestation. So in conclusion, MMP inhibition or dysregulation should manage in such an effective way that would be applicable to concurrent therapies in the world of diseases.

Given current knowledge about MMPs and their correlation with neoplasia, molecular inhibitors of MMPs (MMPIs) were developed and tested in clinical trials. In the early 1990s, MMPIs developed to target cancer showed promise in phase I and II trials, and indeed showed inhibitory effects on growth of both primary and metastatic CRC. However, many phase III trials ended without revealing survival advantages and the use of these agents was associated with substantial toxicity. Synthetic MMPIs were thought to have failed because they lacked selectivity; these agents may have blocked the activity of MMPs that were not over-expressed in a particular cancer or blocked the activity of MMPs with tumor suppressor properties [45].

Another potential reason for the therapeutic failure of MMPIs in these earlier trials was that despite animal studies showing greatest efficacy in early-stage disease, research subjects were enrolled late in their disease course, when they already had metastatic cancer. This point is highlighted by the results of a study involving a synthetic MMPI, MMI270, in a rat model of colon cancer metastasis [46]. MMI270 competitively binds to zinc in the active state of several MMPs including MMP-1, -2, -3, -9 and -13 resulting in their inhibition. Early administration of MMI270, immediately after removing primary colon cancers, significantly reduced lung metastasis compared to delayed administration [77-80].

In the hope of increasing their substrate selectivity, newer structure-based inhibitors of MMPs were developed that take into account the three-dimensional conformation of the enzymatic active site, rather than the previous substrate-based broad-spectrum MMPIs. Other inhibitors of MMPs include SB-3CT which covalently binds to the MMP-2 active site to return the enzyme to its pro-peptide state [47]. An additional group of MMPIs under investigation are chemically-modified tetracyclines (CMTs). Although they lack intrinsic antibiotic activity, their mode of action is thought to involve binding of zinc or calcium ions, or through transcriptional regulation of MMP expression [47].

Another method of targeting MMPs is through microRNAs (miRNAs) that function in transcriptional and post-transcriptional gene regulation. Several of these biological regulators inhibit secretion of MMPs and block MMP activity, thus highlighting their potential as tumor suppressors [4, 27]. In relation with this study, using antisense oligonucleotides to MMP-7 mRNA in human colon cancer cell line xenograft models showed inhibition of basement membrane penetration, and suppression of liver metastases [48]. To further highlight the emerging role of targeting microRNAs in colon cancer, a recent set of experiments were designed to examine the role of miRNA-34a (miR-34a) in human colon cancer.

This miRNA has been shown to be a transcriptional target of p53, a gene that is important in regulating the cell cycle and functions as a tumor suppressor. By transfecting miR-34a into human colon cancer cells, the expression of both MMP-1 and MMP-9 was decreased substantially and ultimately led to inhibition of human colon cancer cell migration and invasion [49]. Although therapeutic inhibition of MMPs previously encountered difficulties, active studies are underway to identify how best to target MMPs in CRC. The role of MMPs as biomarkers of CRC, their use in monitoring therapeutic responses and to identify persons who will likely respond best to a particular chemotherapeutic regimen are all current avenues of investigation. Taken together, based on the role and functions that MMPs play in a host of pathological conditions extending well beyond cancer, the ultimate goal of future work is to develop effective therapy by selective targeting of MMPs.

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Table 1: Prominent MMPs and TIMPs in colorectal cancer (CRC)

MMP/TIMP nomenclature	Actions	Role in CRC	References
MMP-1	Collagenase-1	Expression correlates with CRC invasion and metastasis	[6–8]
MMP-2	Gelatinase A	Expression correlates with CRC invasion	[15–17]
MMP-7	Matrilysin	Expression correlates with CRC cell proliferation, invasion and metastasis	[25–28]
MMP-9	Gelatinase B	Expression correlates with CRC metastasis and is protective in colitis-associated colon cancer	[18–21]
MMP-12	Metalloelastase	Expression correlates with reduced CRC growth and increased survival	[31–34]
MMP-13	Collagenase-3	Expression correlates with diminished CRC survival	[13]
TIMP-1	Inhibits most MMPs	Expression correlates with right-sided CRC and poor survival	[37,38]
TIMP-2	Inhibits MMP-2 and MMP-9	Reduced expression correlates with CRC invasion and worse prognosis	[40,41]
TIMP-3	Inhibits MMPs and ADAMs	Decreased expression correlates with increased CRC invasion	[42]
TIMP-4	Inhibits MMP-2	Expression in rectal cancer correlates with longer survival	[44]

Table 2: Overview of the different mouse and human MMPs.

Name of the MMP	Alternate Name of Enzyme	Localisation	Substrate Specificity
MMP-1	Interstitial collagenase or Collagenase-1	Secreted	Collagens (I, II, III, VII, VIII and X), gelatin, proteoglycan link protein, aggrecan, veriscan, tenacin, entactin
MMP-2	Gelatinase-A, 72 kDa gelatinase	Secreted	Gelatin, Collagens (I, IV, V, VII, X, XI and XIV), elastin, fibronectin, laminin-1, laminin-5, galectin-3, aggrecan, decorin, versican, proteoglycan link protein, osteonectin
MMP-3	Stromelysin-1	Secreted	Collagen (II, IV, V, VII, IX, X, XIV), gelatin, elastin, aggrecan, fibronectin, versican, osteonectin
MMP-7	Matrilysin, PUMP-1	Secreted	Fibronectin, laminin, Collagens(IV, X), gelatin
MMP-8	Neutrophil collagenase	Secreted	CollagenI, II, III, VII, VIII, X, aggrecan, gelatin
MMP-9	Gelatinase-B, 92 kDa gelatinase	Secreted	Gelatin, Collagens (IV, V, VII, X,and XIV), elastin, galectin-3, aggrecan, fibronectin, versican, entactin, osteonectin
MMP-10	Stromelysin-2	Secreted	Collagen IV, laminin, fibronectin, elastin
MMP-11	Stromelysin-3	Secreted	Collagen IV, fibronectin, laminin, aggrecan
MMP-12	Metalloelastase	Secreted	elastin, fibronectin, Collagen IV
MMP-13	Collagenase-3	Secreted	Collagens(I, II, III, IV, IX, X, XIV), gelatin
MMP-14	MT1-MMP	Membrane-Associated	Gelatin, fibronectin, laminin
MMP-15	MT2-MMP	Membrane-Associated	Gelatin, fibronectin, laminin

MMP-16	MT3-MMP	Membrane-Associated	Gelatin, fibronectin, laminin
MMP-17	MT4-MMP	Membrane-Associated	Fibrinogen, fibrin
MMP-18	Collagenase-4, xcol4, xenopus collagenase	-	No known substrate
MMP-19	RASI-1, occasionally referred to as stromelysin-4	-	Gelatin
MMP-20	Enamelysin	Secreted	Amelogenin
MMP-21	X-MMP	Secreted	ND
MMP-23A	CA-MMP	Membrane-Associated	ND
MMP-23B	-	Membrane-Associated	ND
MMP-24	MT5-MMP	Membrane-Associated	ND
MMP-25	MT6-MMP	Membrane-Associated	ND
MMP-26	Matrilysin-2, Endometase	-	ND
MMP-27	MMP-22, C-MMP	-	ND
MMP-28	Epilysin	Secreted	ND

ND-Not detected

Table 3: Overview of the general classification and characteristics of four different human TIMPs.

Property	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Glycosylation	Yes	No	Partial	No
p*I*	8.47	6.48	9.14	7.21
No. of residues[a]	184	194	188	194
M_r[b]	~29	~22	21.7	22.3
MMP inhibition	Weak for MMP-14 -16, -19, and -24, Specific for MMP-9, MMP-1	All, Very specific for MMP-2	All	Most
Other MMP inhibition	ADAM-10	ADAM-12	ADAM-10, -12, -17, -28 and -33; ADAMTS-1, -4, and -5, ADAMTS-2	ADAM-17[d] and -28, ADAM-33

			(weak)	(weak)
Pro-MMP interactions	Pro-MMP-9	Pro-MMP-2	Pro-MMP-9 and pro-MMP-2	Pro-MMP-2
Other partners	CD63 and LRP-1 (MMP-9 complex)	$\alpha_3\beta_1$ integrin LRP-1	EFEMP1, VEGFR2 and Angiotensin II receptor	
Apoptotic effects	Negative	Positive Negative	Positive	
Angiogenesis	Negative	Negative	Negative	Negative
Chromosomal location: human	X11p11.23–11.4	17q23–25	22q12.1–q13.2	3p25

[a]Mature protein.
[b]Excluding any glycans.
[c]Nested in intron V of the listed synapsin.
[d]Inhibited by N-terminal domain of TIMP-4.

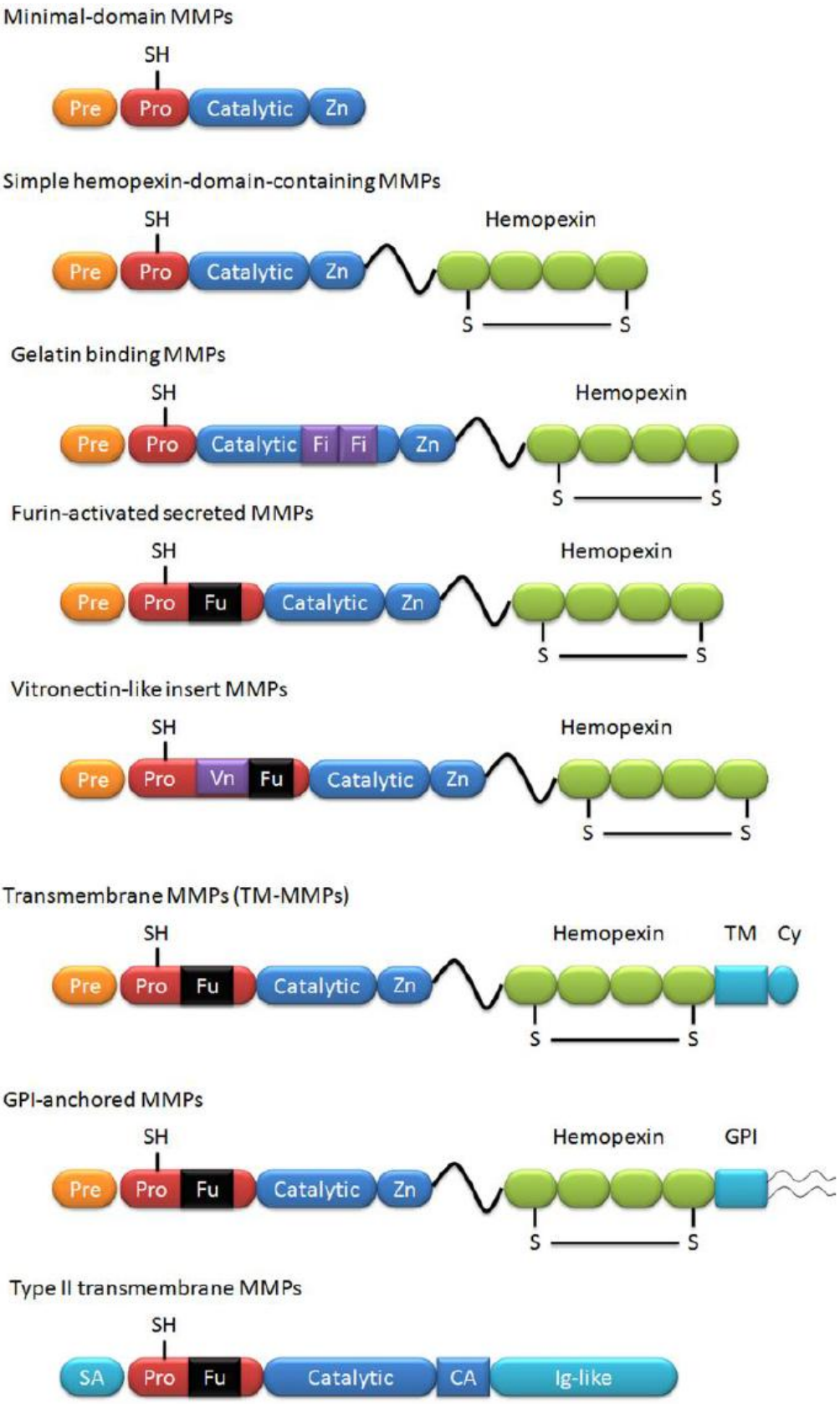

Fig. 1: Structural domains of matrix metalloproteases (MMPs) MMPs are assigned to eight classes on the basis of their structural characteristics, five of which are secreted and three are membrane-type MMPs (MT-MMPs). From the N-terminus, MMPs contain the *Pre*, pre-peptide; *Pro*, pro-domain containing a highly conserved sequence with a cysteine thiol group (*SH*) that interacts with zinc, and maintains the enzyme as inactive zymogen; *Catalytic* domain with a zinc (*Zn*) binding site; Hemopexin-like domain (*Hemopexin*) linked to the catalytic domain by a *Hinge* (∿), which mediates interactions with tissue

inhibitors of metalloproteases, cell surface molecules and substrates. The first and the last hemopexin-like repeats are linked by a disulphite bond (*S-S*). A recognition motif for intracellular furin-like serine proteases (*Fu*) may be present between the pro-peptide and the catalytic domain, and the gelatin-binding MMPs contain inserts that resemble collating-binding repeats of fibronectina (*Fi*). Other inserts are present in MT-MMPs: a single-span transmembrane domain (*TM*), a very short cytoplasmic domain (*Cy*), and the glycosylphosphatidylinositol anchor (*GPI*). In Type II MT-MMPs, an N-terminal signal anchor (*SA*), an unique cysteine array (*CA*) and an immunoglobulin-like (*Ig-like*) domain are also present. Adapted from ref erence 146.