Abstract. The family of human matrix metalloproteinases (MMPs) comprises several tightly regulated classes of proteases. These enzymes and their specific inhibitors play important roles in tumour progression and the metastatic process by facilitating extracellular matrix degradation. As scientific understanding of the MMPs has advanced, therapeutic strategies focusing on blocking these enzymes by matrix metalloproteinase inhibitors have rapidly developed. Low molecular weight tissue inhibitors of matrix metalloproteinase (TIMPs) represent a new therapeutic approach for the treatment of individual types of cancer. This paper aims to briefly summarize current knowledge about the role of MMPs in select non-tumorous lesions, tumor invasion and metastasis. The perspectives in therapeutic intervention in cancer are also mentioned. The role of MMPs in diagnosis and prognosis of colorectal and thyroid cancer is discussed in detail.

The matrix metalloproteinases (MMPs) are a large family of structurally and functionally related endoproteinases (proteolytic enzymes) that are collectively capable of degradation of the basement membrane with subsequent invasion of malignant cells into the host stroma, intravasation into the blood or lymphatic circulation, and formation of metastases in distinct sites (1-9). There is growing evidence, however, that the MMPs have a more expansive role, as they influence many biological pathways during developmental and physiological processes (6, 10), are important for the creation and maintenance of a microenvironment facilitating growth and spreading of neoplasms (5, 6, 9, 11-13), as well as contributing to non-tumorous diseases such as cardiovascular disease.

MMPs are produced by a variety of cell types, including epithelial cells, fibroblasts and inflammatory cells (3). The normal and pathological processes in which MMPs are implicated are listed in Table I (10).

An increasing knowledge of processes and enzymes that are necessary for tumor progression has resulted in a dramatic expansion of potential targets for therapeutic intervention (5-8).

Nomenclature of the MMPs. Metal binding proteinases represent a relatively large and ever growing group of enzymes (5, 10, 14). Rawlings and Barrett have proposed dividing this class of MMPs into clans (based on similarity of protein fold) and families (based on evolutionary relationships) (10). Currently, the MMP class comprises eight clans and some 40 families. The MMP family is a continually growing group, now comprising more than 20 enzymes (1, 10, 14-16).

There are two classification systems of the MMPs: (a) the numbering system, where each enzyme has both a descriptive name (e.g. interstitial collagenase, an enzyme found in the interstitial space, which degrades fibrillar...
Mammalian MMPs are classified into soluble (secreted) type and membrane type (MT-MMP) with respect to this structure based on the presence of a transmembrane domain. Based on substrate specificity, the soluble MMPs can be further classified into collagenases, stromelysins, gelatinases, matrilysins and others as shown in Table II (3, 17, 19).

Characteristics of the MMPs

**Secreted-type subset of MMPs.** These are synthesized in a latent form and are secreted as inactive proMMPs which require extracellular activation. This activation is accompanied by tissue or plasma proteinase cleavage of the cysteine-zinc (known as the cysteine switch) interaction included in the N-terminal propeptide. This process often consists of a two-step reaction. An initial cleavage at the propeptide region leads to removal of the N-terminal polypeptide followed by the second step, an autoproteolytic reaction that generates the stable active enzyme (1, 6, 10, 20). Activation of most MMPs via the cysteine switch mechanisms occurs outside the cell, but several MMPs can be activated before reaching the cell surface. Most MMPs can also be activated by other MMPs or by serine proteases (6, 10, 19, 21).

**Membrane-type subset of MMPs.** These contain a transmembrane domain. Unlike the other members of the MMP family, MT-MMPs are not secreted but remain attached to cell surfaces (1, 5, 19, 22). According to the membrane-anchoring mechanisms, MT-MMPs can be further divided into type I transmembrane MMPs (MT1to3- and MT6-MMP), and type II transmembrane MMPs (MMP-23) (19).

MMP activity can be controlled at different levels (1, 4, 5): transcription, proteolytic activation of the zymogen form, and inhibition of the active form (5, 6). The most important natural inhibitors are tissue inhibitors of MMPs (TIMPs). 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 (6, 20).

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 (1, 5). It is thought that the balance between activated MMPs and TIMPs determines overall MMP activity and proteolysis *in vivo* (4, 14). TIMPs differ in their specificity towards MMPs and in their expression pattern (1, 6, 10, 19, 23, 24).

Role of MMPs in Pathological Conditions

**Matrix metalloproteinases and cardiovascular diseases.** MMPs are proposed as being important contributors to the pathogenesis of cardiovascular disease by remodelling tissues in myocardial fibrosis, blood vessel wall thickening (25) and plaque rupture (26). The majority of acute clinical manifestations of atherosclerosis are due to the physical rupture of advanced atherosclerotic plaques. Endogenous MMPs may be partly responsible for degradation of the fibrous cap of atherosclerotic plaques composed predominantly of type I and III collagen (27, 28). Proteolytic mechanisms causing plaque disruption may result in thromboembolism, coronary artery disease complications and stroke. Increased levels of MMPs are recognized as important risk factors and are associated with worse prognosis of patients with acute coronary syndrome.

Experimental evidence acquired *in vitro* and *in vivo* suggests that the major drives of vascular remodeling, hemodynamics, injury, inflammation and oxidative stress, regulate MMP expression and activity associated with increased atherosclerotic plaques degradation and destabilization. Therefore, any pharmacotherapy aimed at plaque stabilization should target both of these enzymes (27, 28). Statins reduce (*in vitro* and *in vivo*) expression of MMPs.
and tissue factor and reduce acute thrombotic complications of atherosclerosis. Plaque stabilization induced by statins is a complex process caused at least by: short-term anti-inflammatory effect, antithrombotic effect, reduced plaque hemorrhagy, inhibition of MMP-9 and MMP-2, and increase of TIMP-1 (21).

Matrix metalloproteinases in neoplasms. The proteolytic activity of MMPs was championed by Liotta et al. in the early 1980s as one of essential steps of tumor invasion (29). Tumor invasion is considered to be a dynamic, complex, multi-step process coordinated by tumor host interactions. This process is similar for all tumour types and includes disruption of the basement membrane with subsequent invasion of malignant cells into the host stroma, neovascularization for further tumor growth, intravasation into the blood or lymphatic circulation, survival and transport within the circulation, extravasation at distant sites, and growth within the new environment (1, 5, 29).

MMPs have been shown to fulfil other roles in tumor biology: they not only facilitate the breakdown of the extracellular matrix (ECM), but also affect early carcinogenesis events, tumor development, growth and neovascularisation. Invasion or metastasis-facilitating effects of MMPs have been demonstrated using in vitro invasion assays of tumor metastasis in animal models (5, 19, 30, 31). MMPs are expressed both in tumor cells and in neoplastic stromal cells, including predominantly fibroblasts and inflammatory cells. Matrilysin (MMP-9) produced by normal and tumors epithelial cells only represents the exception to this rule (5). MMPs have been intensively studied to elucidate diagnosis and prognosis in some tumours. Owing to a positive correlation between most tumor aggressiveness and the expression of high levels of multiple MMP family members MMPs may serve as diagnostic and/or prognostic tumor markers (1). Numerous studies have been made investigating expression of MMPs including various tumors of the breast (1, 6, 30, 31), lung (1, 11), odontogenic tumors (14), malignant tumors of salivary gland (11), pancreas (1, 11, 24), primary neuroendocrine carcinoma of the skin (4), ovarian tumors (9), prostate cancer (1, 11), and thymic epithelial tumors (13, 22).

Tumor markers are divided according to methods of their study into tissue and biochemical markers, respectively. Tissue markers are studied immediately within tumorous tissue, while biochemical markers may be evaluated after their release into body fluids. Both types of tumor markers have both advantages and limitations.

A comparison of both groups of tumor markers is summarized in Table III. The great advantage of biochemical markers seems to be possibility for their quantifications and monitoring the changes in their
enzymes. The importance of different types of antigens, including those of MMPs and TIMPs to the invasive properties and the malignant potential of the tumors, and therapeutic strategy in cancer therapy.

**MMPs and colorectal cancer.** Colorectal carcinoma (CRC) incidence varies considerably throughout the world, being one of the leading types of cancer in developed countries. Thirty to 60% of patients with CRC undergoing primary surgery with curative intention still die from metastatic disease. Several clinical factors are routinely employed for assessing individual CRC prognosis, such as the pathological state, histological type, grade, vasoinvasive status of the tumor and the status of the resections margin. However, these parameters are insufficient to predict the evolution of each individual patient. Hence finding tumor markers able to differentiate subtypes of early CRC is of high priority to allow the selection of patients with bad prognosis to receive an earlier adjuvant therapy, or radical resectability of colorectal liver metastases, because only this type of surgery is the most effective treatment (33, 34). MMPs and TIMPs are overexpressed in a variety of cancer tissue including colorectal tumors (35). MMP-1 (interstitial collagenase), MMP-2 (gelatinase A), MMP-7 (matrilysin) and MMP-9 (gelatinase B) are overexpressed in colon and rectal cancer cells with respect to normal tissue, and their immunolocalization increases from tubular adenomas to adenocarcinomas (36).
A variety of prognostic factors have been identified to predict the behaviour of colorectal cancer, but these factors are not considered to be susceptible to or modifiable by direct therapeutic intervention. In contrast, MMP-2, which is a prognostic factor in colorectal cancer, could be a target for therapeutic intervention using MMP inhibitors or antibodies.

Quantification of specific mRNA by RT-PCR demonstrated that expression of MMP-2 and its tissue inhibitor, TIMP-2, is different in metastases and normal tissue, and that the active form of MMP-2 is located only in tumoral tissue (37).

It was found that MMP-9 and MMP-7 are overexpressed in colorectal tumor tissue (35, 38). In colorectal patients, MMP-7 expression is significantly correlated with the presence of lymph node or distant metastases; in addition, its enzymatic activity is directly related to the number of metastatic lesions (39). The presence of lymph node metastasis is one of the most important prognostic factors in colorectal cancer, but conventional pathological screening often fails to detect this type of metastasis. Ichikawa et al. successfully detected regional lymph node micrometastases in colon cancer patients using an RT-PCR assay for MMP-7 (40).

Among the MMPs, both MMP-2 and MMP-9 exhibit type IV collagenase activity. Matsuyama et al. reported that MMP-2 and MMP-9 expression in primary tumors is associated with liver metastases in colon carcinomas. In addition, the balance of activity between MMP-2, MMP-9 and TIMP-2 may be relevant to carcinoma invasion and metastasis, including liver metastases in colon carcinoma (41).

TIMPs (21-30 kDa) are the major endogenous regulators of MMP activities. Four homologous TIMPs (TIMPs-1 to -4) have so far been identified (42). TIMP-1 and TIMP-2 are both known to have growth factor-like properties (erythroid potentiating activity), which have been separated from their MMP inhibitory functions by structure function studies (42).

The activity of MMPs depends on the balance between the levels of the active enzyme and its respective TIMP. Tien et al. studied the role of MMPs in colorectal tumor tissue and the metastatic process in the liver. In conclusion, they declared that increased MMP-9 activity could facilitate the hepatic metastatic process in the step after intravasation but not during or before intravasation (43). The relation between the expression of MMP-7 and MMP-9 and the aggressiveness of a colorectal tumor which is connected with a metastatic process was described by Heslin et al. (44).

Waas et al. examined the relationship between MMP activity and tumor recurrence. Both the level of the active forms and the proforms of MMP-2 and MMP-9 were raised in the metastatic tissue of a tumor that recurred in the liver within 6 months after essentially curative liver metastasectomy (45).

Ishida et al. examined serum levels of MMP-9 in portal and peripheral vein blood, and found that increased levels of this MMP correlated with a high risk of hepatic recurrence of colorectal cancer. Assessment of serum MMP level was thus suggested as an useful tool for the selection of patients endangered by hepatic recurrence of colorectal cancer (46). The study by Curran and co-workers is of great interest and contains an MMP/TIMP profile defined by hierarchical cluster analysis of the immunohistochemical score (MMP-7, MMP-9, TIMP-1 and TIMP-2 were included). This study has identified that the MMP/TIMP profile is an independent indicator of poor prognosis and shortened survival rate of patients with colorectal cancer (47).

TIMP-1 inhibits the proteolytic activity of MMPs-7 and -9. But TIMP-1 also fulfilled other functions, such as the stimulation of cell growth and inhibition of apoptosis, which are independent of antiproteolytic activity. In this context, it is not surprising that investigators observed increased levels of expression of mRNA TIMP-1 and also protein TIMP-1 in colorectal carcinoma tissue (48-50). Holten-Andersen et al. reported that high preoperative plasma levels of protein TIMP-1 were associated with a shorter survival of patients with colorectal cancer (51). Waas and co-workers concluded that the preoperative plasma proMMP-2, -9 and TIMP-1 levels had no potential value as diagnostic or prognostic markers in CRC liver metastatic disease (45). Pesta et al. described statistically significant differences in the levels of MMP-2, MMP-7, TIMP-1 and TIMP-2 mRNA between normal colorectal tissue and tumor tissue (52).

**MMPs and thyroid gland tumours.** The majority of thyroid neoplasms arise from follicular epithelial cells and these which are malignant usually belong to the group of the well-differentiated tumors (Table IV). This group of tumors embraces follicular adenoma, minimally and widely expansive follicular carcinoma, and papillary carcinoma. Differential diagnosis between these tumors is based on distinct criteria of malignancy, such as capsular and vascular invasion for follicular adenoma and nuclear features for papillary carcinoma, respectively. Poorly
differentiated carcinomas of follicular cell origin as well as tumors originating from non-epithelial cells are of lower frequency.

Owing to diagnostic and prognostic pitfalls in some cases, thyroid tumors are currently subjected to intensive studies of tumor markers to facilitate the diagnostic process and predict clinical outcome (53-57). MMPs, having a critical role in invasion of the basement membrane, destruction of ECM components, angiogenesis and the metastatic process, should be promising tumor markers in differential diagnosis, particularly between follicular adenoma and carcinoma. The biological behaviour of malignant thyroid tumors may result from pathological expression of MMPs and TIMPs. Several studies have documented the detection of MMPs in thyroid nodular lesions in literature. Maeta et al. investigated the protein expression of MMP-2, MMP-9 and TIMP-1 and TIMP-2 in 86 papillary thyroid carcinomas using immunohistochemistry, semiquantitative scoring morphometry of immunohistochemistry, gelatine zymography and Western blotting (58). Compared with non-tumour regions, these four proteins tended to be overexpressed in the tumour cells: the overexpression was found in 64 of 86 (74%), 80 of 86 (93%), 79 of 86 (92%) and 64 of 86 (74%) cases for MMP-2, MMP-9, TIMP-1 and TIMP-2, respectively. Gelatin zymography showed distinct bands of MMP-2 and MMP-9 in tumor extracts but vague bands in non-tumour extracts. Western blotting revealed the specific bands of MMP-2 and MMP-9 in both tumour and non-tumour extracts. Morphometric scoring revealed that high expression of these proteins significantly correlated with large tumor size, the presence of lymph node metastasis, high clinical stage, high intrathyroidal invasion and high vascular invasion. These data suggest that MMP-2, MMP-9, TIMP-1 and TIMP-2 proteins and activities are increased in tumor cells of papillary thyroid carcinomas and that they play an important role in the invasion and metastasis of papillary thyroid carcinomas (58).

Patel et al. used immunohistochemistry to determine the expression of MMP-1, MT1-MMP, and TIMP-1 in 32 papillary thyroid carcinomas (PTC), 10 follicular thyroid carcinomas (FTC) and 13 benign thyroid lesions from children and adolescents (59). Average MMP-1 expression was significantly greater among PTC and FTC compared to benign lesions, but there was no relationship between MMP-1 expression and invasion, metastasis, or recurrence. Expression of MT1-MMP and TIMP-1 was similar for benign and malignant lesions, but recurrent PTC expressed lower levels of TIMP-1 when compared to non-recurrent PTC. They concluded that MMP-1, MT1-MMP and TIMP-1 are all expressed by thyroid carcinoma and could be important in promoting recurrence (59).

In another study Korem et al. measured MMP protein content and activity in 33 cases of thyroid tumor (papillary, follicular and medullary carcinoma, follicular adenoma and multinodular goiter) by enzyme-linked immunosorbent assay and gel zymography. Immunohistochemistry was also performed (2). They found that the thyroid tissue secreted MMP-2 and -9 as well as TIMP-2, but only MMP-2 was significantly higher in papillary carcinoma compared to the adjacent normal tissue or to the other tumor entities. Increased MMP-2 immunohistochemical staining was demonstrated in the neoplastic papillary epithelial component. No significant difference was seen between papillary carcinomas with lymph node metastases and those without. Thus, increased MMP-2 expression may be useful as a diagnostic marker to differentiate papillary carcinoma from other thyroid neoplasms, but it cannot serve as a useful prognostic marker (2).

Cho Mar et al. (18) have recently reported results of their study where they immunohistochemically analysed MMP-1, MMP-2, MMP-7, MMP-9, MT1-MMP and TIMP-2 in six widely invasive follicular carcinomas (WIFCs), 15 minimally invasive follicular carcinomas (MIFCs), 19 follicular adenomas (FAs) and 10 adenomatous goiters (AGs). MMP-1 was found in all follicular thyroid lesions (FTLs). MMP-2 and MMP-7 were positive in more than 80% of WIFC and MIFC cases, whereas they were absent from all FA and AG cases except one MMP-2 in FA. MMP-9 staining was significantly more positive in MIFC than FA or AG cases. Their results confirmed MMP expression mainly in malignant FTLs (18). Their results on MMP-2 are in accordance with those of Campo et al. (60), who reported that MMP-2 expression was associated with tumour invasion and metastasis in thyroid papillary and follicular carcinomas, the latter including a few MIFCs. Their results are also supported by the study by Pasieka et al. (61) which demonstrated a higher concentration of MMP-2 and TIMP-2 in the serum of patients with malignant compared with benign thyroid tumours. The authors believe the MMP-2 and MMP-7 are useful markers to distinguish MIFC from FA.

Inhibition of MMPs in cancer therapy. The crucial role of the MMPs in tumor progression and metastasis has initiated intense development of therapeutic agents blocking enzyme activity and thus inhibiting tumor progression (30). One of the first low molecular weight MMP inhibitors to be tested in patients was batimastat, a potent broad-spectrum agent blocking MMP-1, MMP-2, MMP-3 and MMP-9. Marimastat is a second-generation synthetic MMP inhibitor, structurally similar to batimastat, which is water-soluble and can be given orally. Similar effects in primary tumor growth and the number and size of metastases have been demonstrated with other synthetic MMP inhibitors (1, 8).

Several generations of synthetic TIMPs were tested in phase III trials in humans, which include synthetic collagen peptidomimetic MMP inhibitors, synthetic tetracycline derivates (Metastat), COL-3 (8) and biphosphonate...
inhibitors (6). The results of these clinical trials have been disappointing as no therapeutic efficacy was seen. One of the reasons for the failure of TIMPs in human trials has been the fact that many analyses have been performed in advanced stage tumors when malignant cells have already undergone metastatic transformation, while TIMPs may be more important in the early stages of cancer due to their cytostatic rather than cytotoxic effect (6).

**Conclusion**

Large amounts of data have been obtained relating to MMP overexpression in various tumor types when compared to normal tissue, and several studies have provided evidence that certain MMPs in specific types of cancer can be useful as diagnostic and prognostic tumor marker indicators of disease progression. MMPs seem to be promising diagnostic and/or prognostic markers namely in thyroid and colorectal cancer. Prognosis of CRC was proven to be dependent on the extent of local and metastatic tumour spread, and the degradation of the extracellular matrix surrounding the tumour cells is a key step in tumour invasion. As mentioned above, the main group of enzymes involved in matrix degradation are the MMPs dependent on their relation to TIMPs.

Matrix metalloproteinases are also considered to be promising targets for cancer therapy due to their strong involvement in malignant pathologies. On this point, the role of the pathologist in evaluation of the expression of MMPs and TIMPs within tumors alone is indisputable. A better understanding of the expression and pathobiology of MMPs and their inhibitors in individual malignant tumors may help to change current cancer therapy towards more specific and patient-friendly approaches.

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