Matrix metalloproteinases in gastrointestinal cancer

Matrix metalloproteinases (MMPs) are a family of enzymes that are responsible for the breakdown of connective tissue proteins. These enzymes play a central role in tissue remodelling associated with growth, development and repair. In these physiological processes MMP activity is tightly regulated. However, it is clear that aberrant MMP expression can contribute to the pathogenesis of several diseases including rheumatoid arthritis, tumour invasion and metastasis, multiple sclerosis, cerebral haemorrhage, and inflammatory bowel disease. The focus of this short article is the expression of MMP activity in gastrointestinal cancer. Rather than provide a complete overview of research in this area the article will consider areas of particular clinical relevance, such as the potential use of MMPs as prognostic markers and the use of MMP inhibitors in the treatment of cancer. For further insight into the role of MMPs in tumour invasion and metastasis the reader is referred to the recent review by Chambers and Matrisian.

Matrix metalloproteinases

Sixteen MMPs have been identified to date (MMPs 1–19, numbers 4–6 were not used). The enzymes are structurally related and take their name from a zinc atom in the active site. The enzymes range from the well characterised interstitial collagenase (MMP-1) which degrades fibrillar collagens, to the more obscure membrane-type (MT-) MMPs whose functions and substrates are still largely to be defined. As might be expected for enzymes with such destructive potential the activity of MMPs is tightly regulated at several levels. Some MMPs do seem to be expressed constitutively in normal tissues (for example, progelatinase A in vascular smooth muscle) but in general their expression is linked to active processes of tissue remodelling such as trophoblast implantation or mammary gland involution. The enzymes are secreted in an inactive proform with an amino-terminal domain blocking the active site. In most cases removal of the pro-domain and consequent activation occurs extracellulary. The mechanisms of activation are not completely understood although MT1-MMP (MMP-14) does seem to be specifically responsible for the activation of progelatinase A (MMP-9). Once activated MMPs are subject to further control by a group of inhibitors known as “tissue inhibitors of metalloproteinases” (TIMPs), of which four have been identified to date.

In malignancy MMPs seem to be induced and used by invasive tumours to remodel the local environment, allowing both tumour growth and the development of a supportive network of new blood vessels. Furthermore, the capability of MMPs to degrade vascular basement membranes indicates a potential to facilitate metastasis.

MMP expression in human gastrointestinal cancer

One of the features of studies on MMP expression in cancer is that the results often seem to conflict with one another. This can lead to heated debates among MMP researchers and general confusion for those working outside the field. Many of the problems arise with studies of small series of samples or with the application of different techniques. For example routine immunohistochemical analyses do not differentiate between latent and activated forms of MMPs, such that an analysis of gelatinase A expression may fail to show a relation with tumour stage or survival whereas a study of the expression of activated gelatinase A by substrat gel electrophoresis (zymography) might do so. Different cellular localisations for a particular MMP have also been reported. This may be due to differences between the epitopes recognised by different antibodies. In addition, the location of MMP mRNA as detected by in situ hybridisation may be more discrete than the detection of MMP protein detected immunologically.

Despite these difficulties some general observations can be made. Several MMPs are expressed in gastrointestinal cancers at levels that are higher than those found in normal tissue or benign adenomas. In many studies the extent of MMP expression has been related to tumour stage, with stage IV tumours showing the highest levels. Levels of interstitial collagenase, gelatinase A, gelatinase B (MMP-9), matrilysin (MMP-7), stromelysin-3 (MMP-11) and MT1-MMP are elevated in colorectal cancer and those of gelatinase A, gelatinase B, matrilysin and MT1-MMP are elevated in gastric cancer. Expression of gelatinase A, gelatinase B, stromelysin-3 and matrilysin has also been detected in colorectal liver metastases.

It is not clear at what point in tumour progression MMP activity rises. In an elegant series of experiments Wilson et al have shown that in mice that are genetically prone to multiple intestinal neoplasms (Min+/−), deletion of matrilysin by targeted mutagenesis results in a 60% reduction in mean tumour multiplicity compared with wild type Min/+ mice. This suggests a role for matrilysin in early tumorigenesis.

The source of MMPs in human cancer was originally assumed to be the carcinoma cells. However, microdissection techniques and mRNA in situ hybridisation have
revealed a more dynamic picture of MMP expression. It seems that the expression of several MMPs is induced in stromal tissue, with highest levels of induction at the invasive margins. This was first noted for stromelysin-3 expression in breast cancer and has subsequently been observed with gelatinase A and stromelysin-3 expression in colorectal cancer. MT1-MMP mRNA has been detected in both carcinoma cells and associated stromal cells. In gastric carcinoma MT1-MMP co-localised with gelatinase A in invasive tumour nests. Zymography of microdissected nests showed activated gelatinase whereas only latent pro-gelatinase was detected in other parts of the tumour.

This suggests a process whereby gelatinase A is induced in tumour associated stromal tissue and is then activated by MT1-MMP on the tumour cell surface. In situ hybridisation studies have also shown the production of gelatinase B by tumour associated macrophages and the production of matriptase by colon carcinoma cells.

One of the consequences of the interaction between tumour and stroma in the production and activation of MMPs is that the pattern of expression is likely to be site dependent. Our own studies have shown that activated gelatinase A is detected at high levels in primary breast cancer samples but is not detectable in lymphatic metastases from the same patients. The expression of MMPs in hepatic metastases from colorectal primaries suggests that the interaction between stroma and tumour in this organ is permissive for MMP mediated invasive growth. If MMPs are actively involved in cancer invasion and metastasis then one might expect MMP levels to be of some prognostic significance. When patients are considered across tumour stage, an association between high levels of MMP expression and shorter survival can be established. However, within a particular tumour stage MMP expression has not been convincingly shown to be of prognostic value. An immunohistochemical study of interstitial collagenase in colorectal cancer determined that this MMP was a significant predictor of overall survival independent of Dukes’ stage but only 10 of 64 samples tested were immunopositive. This is difficult to reconcile with earlier immunohistochemical studies in which collagenase positivity was observed in most colorectal tumours.

In a separate study Zeng and colleagues have shown by multivariate analysis that gelatinase B mRNA expression in colorectal cancer was a significant independent predictor of disease-free survival but was not quite significant for overall survival. However, this result is qualified by the use of a statistical technique to reach an optimised cut off point which defined 41 of the 71 patients as having “high” levels of gelatinase B expression. Similarly, Seir and colleagues have shown that in gastric cancer activated gelatinase A and progelatinase B are significant prognostic factors independent of standard clinicopathological parameters such as Lauren classification, or tumour size. Again an optimised cut off point was used with 23 of 50 patients being defined as having “high” levels of activated gelatinase and 17 of 50 patients as having high levels of progelatinase B. Elevated plasma concentrations of MMPs have also been detected in patients with gastrointestinal cancer but an independent prognostic value for these levels has not been established.

TIMP-1 levels have also been shown to be elevated in cancer. This has been observed in both colorectal and gastric neoplasms, and elevated TIMP-1 expression has been linked to metastatic spread and poor prognosis. Although this seems to run against the hypothesis that MMP activity promotes tumour invasion, it may reflect the need for some regulation of the increased metalloproteinase activity. Clearly, to “succeed” a malignant neoplasm must do more than degrade its immediate environment. In effect the tumour must “remodel” the local tissue to suit its own needs and this would require increased levels of both proteases and inhibitors.

In summary, it is possible that further studies may yet reveal diagnostic and prognostic applications for MMPs or TIMPs. However, these studies will need to be larger and the cut off points between high and low expression should be prospectively defined.

**Therapeutic use of MMP inhibitors**

There has been a long standing interest in the use of proteinase inhibitors in the treatment of cancer. As more has become known about the involvement of MMPs in tumour invasion and metastasis, the objectives of inhibitor treatment have developed from the simple inhibition of metastasis to include a more comprehensive suppression of both primary and secondary tumour growth. This broader therapeutic goal is supported by results of experiments with MMP inhibitors in animal cancer models.

Both native inhibitor proteins such as TIMP-1 and low molecular weight synthetic inhibitors have been considered as potential treatments. Synthetic inhibitors have been easier to develop as they can be designed to have different spectra of activity against members of the MMP family and can be modified to be orally bioavailable. Many of the early synthetic inhibitors were derived from the peptide structure at the point in the collagen molecule which is first cleaved by interstitial collagenase. More recently non-peptide inhibitors have been designed with the aid of x-ray crystallographic structures of the MMP active site. A common feature of these inhibitors is the presence of a metal binding group, frequently hydroxamic acid, which is positioned to chelate the active site zinc atom. The compounds are potent reversible inhibitors of the MMP family but generally show little or no activity against metalloproteinases outside of this group.

The broad spectrum MMP inhibitor batimastat (BB-94) was one of the first to be tested widely in cancer models. In a xenograft model in which fragments of human colorectal tumour were implanted in the intestine wall of nude mice, batimastat treatment was shown to reduce both tumour growth and loco-regional invasion. A similar result was obtained in this model with the selective MMP inhibitor CT1746 which shows greater potency for gelatinase A and B than for interstitial collagenase and matriptase. This suggests that a more selective inhibitor than batimastat might be equally effective. However, experimental cancer models cannot reproduce the human stroma–tumour interaction and the pattern of MMP expression is likely to differ from that seen clinically where stage related over-expression of both interstitial collagenase and matriptase has been documented.

Batimastat also inhibits the invasive growth of C170HM, human colorectal tumours in nude mice. These tumour cells invade and grow within the liver following intraperitoneal injection. In addition to a reduction in the number and size of the liver tumours, batimastat treatment was associated with a notable increase in the extent of central necrosis. This effect may be due to inhibition of tumour angiogenesis or to increased compression of existing vasculature as expansive growth is prevented. Increases in fibrotic content and deposition of fibrotic capsules have also been observed in some experimental tumours treated with MMP inhibitors, although the histological appearance of other treated tumours is unchanged despite a reduction in growth rate.

Clinical trials of MMP inhibitors in patients with cancer have now started and gastrointestinal malignancies have been among the first studied. As MMP inhibitors are...
intended to be tumourostatic compounds and are not cytotoxic, conventional cytoreductive tumour responses could not be used to evaluate their activity in phase I trials. Instead alternatives of direct observation and the use of surrogate markers have been investigated. Although experimental, such approaches may provide a means of establishing a biologically active dose range for randomised studies. In a trial of the oral inhibitor marimastat (BB-2516) in patients with non-resectable gastric cancer the primary tumours were examined at the beginning and end of a four week treatment period by endoscopy and biopsy. Structural changes were detected in the tumours of some patients consistent with an increase in fibrotic stroma. These changes matched those seen in animal cancer models.

In a second study changes in the rate of rise of carcinoembryonic antigen (CEA) were used as a means of detecting effects in patients with advanced colorectal cancer. Although CEA concentrations are known to fluctuate in patients with advanced colorectal cancer models.31 The results of these studies should show whether inhibitors of metalloproteinases in human colorectal tumours. Jpn J Clin Oncol 1996;26:303–9.


