Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer

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Colorectal cancer is one of the commonest malignant tumors and has a relatively poor prognosis. The outcome depends on the extent of local and particularly metastatic tumor spread. The matrix metalloproteinases (MMPs) are a family of closely related enzymes that degrade the extracellular matrix and are considered to be important in facilitating tumor invasion and spread[1-3]. Using immunohistochemistry we have investigated the occurrence in colorectal cancer of MMP-1 (interstitial collagenase). Our monoclonal antibody was prepared against a synthetic peptide corresponding to an amino acid sequence specific for MMP-1 and was selected to react in formalin-fixed wax-embedded sections, thus allowing use in diagnostic histopathology and also enabling access to archival material. We found that the presence of MMP-1 in colorectal cancer is associated with a poor prognosis ($P = 0.006$) and has prognostic value independent of Dukes stage. One MMP inhibitor that strongly inhibits MMP-1 has already been shown to inhibit growth of human colon cancer xenografts in nude mice[4]. Our results suggest that treatment of those individuals whose colon tumors produce MMP-1 with MMP inhibitors is a therapeutic strategy worth pursuing.

Colorectal tumors ($n = 64$; age range of patients; 38–92; 38M, 26F) submitted consecutively over the period January to June 1991 to the Department of Pathology, University of Aberdeen, for diagnosis were used in this study. All the tumor samples had been fixed in formalin and embedded in wax. Histologically all the tumors were adenocarcinomas and pathological staging of the tumors was performed according to Dukes classification (as follows, Dukes A = 1, Dukes B = 38, and Dukes C = 25). All the patients had survived at least one month following surgery and were followed up for 46–52 months.

Sections of colorectal tumors were immunostained with a monoclonal antibody (3B6) specific for MMP-1. In the production of this antibody we used a synthetic peptide corresponding to a selected region of amino acid sequence for immunization and screening (after attachment to different carrier proteins). This region was chosen because (1) it represents a sequence not found in any other member of the MMP family, (2) it is present in both the active and zymogen forms and (3) it was found by homology modeling to be on the surface of the molecule. A similar rationale had proved successful in distinguishing between the various isozymes of human enolase[5]. Immunoblotting showed that the MMP-1 antibody recognized MMP-1 and did not recognize MMP-2, MMP-3 or MMP-9 (Fig. 1).

Positive immunoreactivity for MMP-1 was identified in 10 (16%) of the tumors, whereas 54 (84%) tumors showed no MMP-1. In the tumors that showed immunoreactivity, more than 90% of tumor cells were positive. There were 8 (80%) deaths in the MMP-1-positive group, and the median survival was 11 months, whereas there were 27 (50%) deaths in the MMP-1-negative group with a median survival of 46 months (Fig. 2). Statistical analysis showed that survival of patients with MMP-1-positive tumors was significantly less than that of patients whose tumors did not show MMP-1 (log-rank test, $P = 0.006$). Five of the MMP-1-positive tumors were Dukes stage B and five were Dukes stage C; the occurrence of MMP-1 remained significant ($P = 0.01$) after multivariate analysis for Dukes stage and patient age, indicating that MMP-1 is an independent prognostic factor in colorectal cancer.

Because there was only one Dukes A tumor in this study we carried out an additional investigation on 12 Dukes A cases. The immunohistochemical results showed no immunoreactivity in eight samples; the other four had a much lower intensity of staining than in the positive samples in the main study. In clinical practice, Dukes A patients do not represent a continuing therapeutic problem, because they are considered to be cured following surgery, presumably because the tumor cells are removed before they can metastasize.

Initial tumor invasion depends on degradation of the basement membrane surrounding individual tumor cells, whereas spread of established malignant tumors depends on digesting interstitial connective tissue. Therefore the action of MMP-1, which degrades collagen types I, II and III, the main types of col-

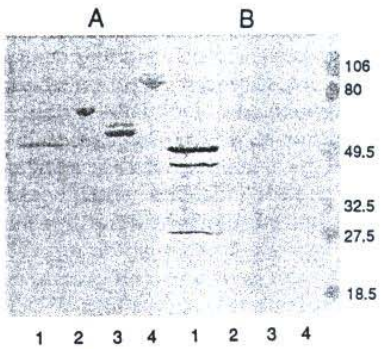

Fig. 1 Immunoblot showing specificity of anti-MMP-1 antibody. Duplicate 1-μg samples of each individual MMP (MMP-1, lane 1; MMP-2, lane 2; MMP-3, lane 3; and MMP-9, lane 4) were subjected to SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to PVDF membrane. The membrane was then divided in two; section A was stained for protein with amido black and section B immunoblotted with the anti-MMP-1 monoclonal antibody 3B6. Prestained molecular weight markers are shown on the right. The antibody reacts only with MMP-1: the three bands in the MMP-1 lane correspond to the proenzyme, the activated enzyme and the carboxy-terminal domain (where the epitope is located), produced by breakdown of the MMP-1 protein.

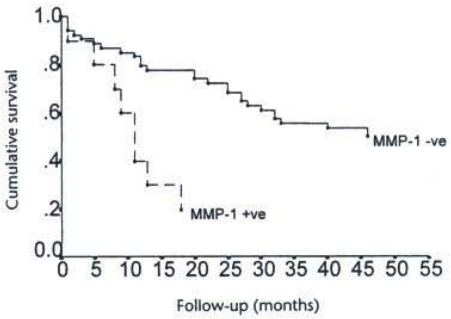

Fig. 2 Survival plots showing the relationship between the occurrence of MMP-1 and survival in patients with colorectal cancer. Survival differed significantly between MMP-1-positive and -negative tumors ($P = 0.006$).

lagen in interstitial connective tissue, is possibly more important in facilitating the spread of established invasive tumors. In this study we have shown that the presence of MMP-1 in colorectal cancer is associated with a significantly poorer prognosis compared with those tumors that do not show MMP-1. Our antibody does not distinguish between the precursor and active forms of MMP-1, but it is likely that we are detecting mainly active enzyme, as MMP-1 is known to have increased activity in colon tumors[6]. These results support the hypothesis that MMP-1 is important for facilitating the spread of colorectal cancer. Increased collagenase activity toward type I (ref. 6, 7) and type III (ref. 6) collagens has previously been identified in colorectal cancer. These collagenase activities correlated with the extent of invasion into muscularis, although no relationship with survival was reported. The presence of increased collagenase has previously been reported from immunohistochemical studies[8]: some variation in tissue localization between their work and ours may result from differences in immunohistochemical methodology. Furthermore, studies of collagenase messenger RNA have also shown increases in colon tumors[9,10], although it is difficult to extrapolate from the identification of mRNA to the presence of protein, particularly when the protein is known to be processed and secreted postsynthetically.

A variety of prognostic factors have been identified to predict the behavior of colorectal cancer[11], but these factors are not considered to be susceptible to or modifiable by direct therapeutic intervention. In contrast, MMP-1 is a prognostic factor in colorectal cancer that could be a target for therapeutic intervention using MMP inhibitors[12] or antibodies. Indeed one MMP inhibitor that markedly inhibits MMP-1 has been shown to decrease the growth of human colon cancer xenografts in nude mice and to prolong their survival[4].

As the monoclonal antibody to MMP-1 used in this study detects MMP-1 immunoreactivity in formalin-fixed, wax-embedded sections, it could be used in routine diagnostic histopathology to evaluate MMP-1 status in colorectal cancers and thus to identify those patients who might benefit from anti-MMP therapy.

Methods

For immunohistochemistry, tissue sections were subject to an antigen retrieval step by microwaving the sections for 20 min in 0.01M citrate buffer, pH 6.0, before application of the MMP-1 antibody, and an alkaline phosphatase anti-alkaline phosphatase method was used to detect sites of immunoreactivity[13]. The slides were examined using light microscopy by two observers (G.I.M. and P.O'N.) in order to determine qualitatively the presence or absence of immunostaining, and its distribution. MMP-1 immunoreactivity in the tumors was determined before obtaining the patient survival data.

Cumulative patient survival was assessed by the method of Kaplan-Meier, and comparison of the MMP-1-positive and MMP-1-negative survival curves was performed using the log-rank test. Cox regression analysis was performed to determine whether MMP-1 was an independent prognostic factor.

Acknowledgments

We thank the Scottish Hospital Endowments Research Trust for financial support and Alan Galloway, British Biotechnology Group plc, for the generous gift of matrix metalloproteinases.

RECEIVED 28 AUGUST 1995; ACCEPTED 8 FEBRUARY 1996

1. Hart I.R. & Saini, A. Biology of tumour metastasis. *Lancet* 339, 1453–1457 (1992).
2. Liotta, L.A., Steeg, P.S. & Stetler-Stevenson, W.G. Cancer metastasis and angiogenesis: An imbalance of positive and negative regulation. *Cell* 64, 327–336 (1991).
3. Stetler-Stevenson W.G., Liotta, L.A. & Kleiner, D.E. Extracellular matrix 6: Role of matrix metalloproteinases in tumour invasion and metastasis. *FASEB J.* 7, 1434–1441 (1992).
4. Wang, X., Fu, X., Brown, P.D., Crimmin, M.J. & Hoffman, R,M. Matrix metalloproteinase inhibitor BB-94 (Batimastat) inhibits human colon tumour growth and spread in a patient-like orthotopic model in nude mice. *Cancer Res.* 54, 4726–4728 (1994).
5. Duncan, M.E., McAleese, S.M., Booth, N.A., Melvin, W.T. & Fothergill, J.E. A simple enzyme-linked immunosorbent assay (ELISA) for the neuron-specific gamma isozyme of human enolase (NSE) using monoclonal antibodies raised against synthetic peptides corresponding to isozyme sequence differences. *J. Immunol. Meth.* 151, 227–236 (1992).
6. Van Der Stappen, J.W.J., Hendriks, T. & Wobbes, T. Correlation between collagenolytic activity and grade of histological differentiation in colorectal tumors. *Int. J. Cancer* 45, 1071–1078 (1990).
7. Irimura, T., Yamori, T., Bennett, S.C., Ota, D.M. & Cleary, K.R. The relationship of collagenase activity to stage of human colorectal tumours. *Int. J. Cancer* 40, 24–31 (1987).
8. Hewitt, R.E. *et al.* Distribution of collagenase and tissue inhibitor of metalloproteinases (TIMP) in colorectal tumours. *Int. J. Cancer* 49, 666–672 (1991).
9. Gray, S.T., Yun, K., Motoori, T. & Kuys, Y.M. Interstitial collagenase gene expression in colonic neoplasia. *Am. J. Pathol.* 143, 663–671 (1993).
10. Urbanski, S.J., Edwards, D.R., Hershfield, N., Huchcroft, S.A., Shaffer, E., Sutherland, L. & Kossakowska, A.E. Expression pattern of metalloproteinases and their inhibitors changes with the progression of human sporadic colorectal neoplasia. *Diagn. Mol. Pathol.* 2, 81–89 (1993).
11. Fenoglio-Preiser, C.M. & Noffsinger, A.E. Epithelial tumours and tumour-like conditions. in *Gastro-intestinal and Oesophageal Pathology* (ed. Whitehead, R.) 863–892 (Churchill Livingstone, Edinburgh, 1995).
12. Brown, P.D. Matrix metalloproteinase inhibitors: A new class of anticancer agent. *Curr. Opin. Invest. Drug* 2, 617–626 (1993).
13. Murray, G.I., Duncan, M.E., Melvin, W.T. & Fothergill, J.E. Immunohistochemistry of neuron specific enolase with gamma-subunit specific anti-peptide monoclonal antibodies. *J. Clin. Pathol.* 46, 993–996 (1993).