J. Biochem. Mol. Biol. Vol. 29, No. 5 pp. 484~487 September 30, 1996

Expression of the Type IV Collagenase Genes and *ras* Oncogene in Various Human Tumor Cell Lines

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(Received July 9, 1996)

Abstract: The matrix metalloproteinases (MMPs) are members of a unique family of proteolytic enzymes that degrade components of the extracellular matrix. Significant evidence has accumulated to directly implicate members of the MMPs in tumor invasion and metastasis formation. To investigate the correlation between *ras* oncogene and MMP gene expression in various tumor cells, we detected mRNAs for the *ras*, MMP-2 and MMP-9 (72 kD and 92 kD type IV collagenases, respectively) genes in nine human tumor cell lines. The *ras* gene was expressed in seven cell lines; MMP-2 in three; MMP-9 in two cell lines tested. There was no direct correlation between the *ras* oncogene and MMP expression. A clear difference in the mRNA expression between MMP-2 and MMP-9 was observed among the cell lines. As an approach to study the effect of the *ras* oncogene on metastasis, we examined the expressions of MMP-2 and MMP-9 in HT1080 cells transfected with the v-H-*ras* gene. MMP-9 expression was significantly enhanced in the *ras*-transfected HT1080 cells compared with the nontransfectants while *ras* transfection did not affect the expression of MMP-2. These results suggest the possible inducing effect of the *ras* oncogene on the metastasis by activation of the MMP-9 gene in HT1080.
Key words: matrix metalloproteinase, metastasis, *ras* oncogene, tumor cell line, type IV collagenase.

Tumor metastasis involves the spread of cancer cells to secondary sites and is responsible for many of the failures in cancer treatment. An essential part of the metastatic process includes degradation of the extracellular matrix and basement membrane, the turnover rate of which is strictly controlled to maintain normal tissue structure as well as the developmental process, uterine involution and wound healing. Uncontrolled degradation of the extracellular matrix and basement membrane is thought to be associated with tumor cell invasion (Liotta, 1986).

The matrix metalloproteinases (MMPs) are a family of enzymes secreted by resident and inflammatory cells that are collectively capable of degrading most or all of the constituent macromolecules of the extracellular matrix. Significant evidence has accumulated to directly implicate members of the gene family of MMPs in tumor invasion and metastasis formation (Liotta *et al.*, 1991). Each MMP has a different substrate specificity. Interstitial collagenase (MMP-1) degrades type I, II, and III collagens and stromelysins degrade proteoglycan core protein, laminin, fibronectin, and gelatin (Matrisan, 1990). The third group of enzymes of the MMP gene family are the type IV collagenases. The 72 kDa and 92 kDa gelatinases/type IV collagenases (MMP-2 and MMP-9, respectively) can degrade type IV and V collagens, fibronectin, laminin and gelatins (Liotta *et al.*, 1980; Fessler *et al.*, 1984). The two enzymes arise from separate mRNA transcripts. MMP-2 was identified in H-*ras* oncogene-transformed human bronchial epithelial cells (Collier *et al.*, 1988) and MMP-9 was characterized in SV40-transformed human lung fibroblasts (Wilhelm *et al.*, 1989). It has been recently demonstrated (Fridman *et al.*, 1995) that the activation of MMP-9 proenzyme was mediated by MMP-2 species that might be localized in the surface of tumor cells.

The effect of known oncogenes on metastasis has been tested. It has been shown that the *src* oncogene can induce the metastatic phenotype (Chambers and Wilson, 1985; Egan *et al.*, 1987). The *ras* oncogene has also been demonstrated to promote metastatic ability (Bondy *et al.*, 1985; Bradley *et al.*, 1986). Induction of the malignant phenotype using the *ras* oncogene has been shown to enhance expression of type IV collagenolytic activity (Garbisa *et al.*, 1987; Collier *et al.*,

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1988; Ura *et al.*, 1989). Sato *et al.* (1992) suggested a correlation between malignant transformation by c-H-*ras* oncogene and enhanced expression of mRNA for MMP-9 in KMS-6 fibroblasts.

In the present study, we examined the mRNA expression of genes responsible for type IV collagen degradation (MMP-2 and MMP-9) and *ras* oncogene in nine human tumor cell lines. We also studied whether the expression of these MMP genes is associated with the *ras* oncogene overexpression in HT1080 fibrosarcoma tumor cell line.

Materials and Methods

Cell lines and culture condition

All the cell lines are of human origin. Fibrosarcoma HT1080 was kindly provided by Dr. Kyu-Won Kim (Department of Molecular Biology, Pusan National University, Pusan, Korea). Hepatocellular carcinomas (SK-Hep-1, Hep G2 and Hep 3B), melanomas (SK-Mel-1 and Malme-3M), gastric carcinoma (Kato-III) and cervix carcinomas (HeLa M_r and HeLa S3) were obtained from American Type Culture Collection. All the cells were cultured at 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics.

DNA transfection

The DNA used was plasmid pras-zip6 which contained v-H-*ras* oncogene inserted in pZipNeo SV (X) expression vector. The DNA was transferred to HT1080 fibrosarcoma cells by liposome-mediated tranfection (Felgner *et al.*, 1987). We used Lipofectin^R (Gibco BRL, Gaithersburg, USA) and followed the manufacturer's technical manual.

Northern blotting

Total RNA was isolated by a modification of the method of Chomczynski and Sacchi (1987). RNA was fractionated by electrophoresis on 1% agarose gel containing 2% formaldehyde and transferred overnight to nylon filter (Hybond N^+, Amersham). To analyze the expression of the MMP-2 or MMP-9 mRNA in tumor cell lines, 1.5 kb *Eco*RI-*Bam*HI fragment or 1.2 kb *Pst*I-*Eco*RI fragment DNA probe was excised from MMP-2 (Collier *et al.*, 1988) or MMP-9 (Wilhelm *et al.*, 1989) cDNA clones, respectively, which were kindly provided by Dr. Kyu-Won Kim. The *ras* gene probe was excised as 0.7 kb *Bam*HI fragment from pras-zip6 plasmid which was also provided by Dr. Kyu-Won Kim. The β-actin gene was used as a house-keeping gene probe. The DNA fragments were labeled with [α-^{32}P]dCTP (3,000 Ci/mmol, Amersham, Buckinghamshire, England) using the multiprime DNA labeling system (Amersham). Northern blot analysis was performed as described in Sambrook *et al.* (1989) with a slight modification.

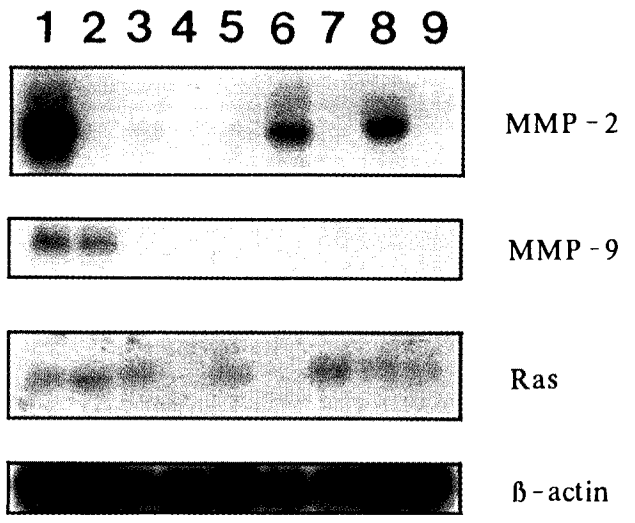

Fig. 1. Expression of mRNAs for MMP-2, MMP-9 and *ras* in nine human tumor cell lines. Methods for Northern blot analysis are described in Materials and Methods. Lane 1, HT1080 fibrosarcoma; lane 2, SK-Hep-1 hepatocellular carcinoma; lane 3, Hep G2 hepatocellular carcinoma; lane 4, Hep 3B hepatocellular carcinoma; lane 5, SK-Mel-1 melanoma; lane 6, Malme-3M melanoma; lane 7, Kato-III gastric carcinoma; lane 8, HeLa M_r cervix carcinoma; lane 9, HeLa S3 cervix carcinoma.

Results and Discussion

Human tumor cell lines originating from mesenchymal cells (HT1080) and epithelial cells (SK-Hep-1, Hep G2, Hep 3B, SK-Mel-1, Malme-3M, Kato III, HeLa M_r, and HeLa S3) were examined for their expression of mRNAs for MMP-2, MMP-9 and *ras* (Fig. 1). The MMP-2 mRNA was detected in three out of nine cell lines tested and MMP-9 in two cell lines. The low percentage of MMP expression (33% for MMP-2 and 22% for MMP-9) indicates that tumorigenicity does not necessitate the expression of MMP gene(s). The MMP-2 mRNA was expressed in HT1080, Malme-3M and HeLa M_r (Fig. 1, lanes 1, 6 and 8, respectively). The highest mRNA expression for MMP-2 was observed in HT1080 fibrosarcoma. It has been shown that tumor cells originating from mesenchymal cells expressed significant levels of mRNA for MMP-2 while its expression was rare in epithelial tumor cells (Sato *et al.*, 1992). Moreover, Collier *et al.* (1988) reported the expression of MMP-2 in fibroblastic cells but not in normal epithelial cells. These findings suggest that the mRNA expression of MMP-2 is not characteristic of tumor cells but appears to be mesenchymal cell type-specific. Expression of MMP-9 was detected in HT1080 and SK-Hep-

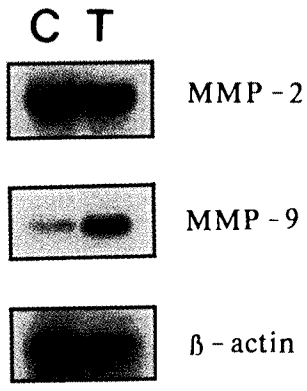

Fig. 2. Expression of mRNAs for MMP-2 and MMP-9 in HT1080 fibrosarcoma cells (C) and HT1080 cells transfected with v-H-*ras* oncogene (T). Methods for transfection and Northern blot analysis are described in Materials and Methods.

1 cells (Fig. 1, lanes 1 and 2, respectively) with similar degrees. A clear difference in the mRNA expression for MMP-2 and MMP-9 was observed among the tumor cell lines tested except for HT1080 cells which expressed both MMP-2 and MMP-9 mRNAs. This result suggests that each cancer cell line has a different kind of type IV collagenase expression. Analysis of transcriptional regulation has revealed that MMP-2 is regulated in a unique and independent manner when compared with other members of the collagenase family, including MMP-9 (Brown *et al.*, 1990). The *ras* gene was expressed in seven cell lines : HT1080, SK-Hep-1, Hep G2, SK-Mel-1, Kato-III, HeLa M_r and HeLa S3 (Fig. 1, lanes 1-3, 5, 7-9, respectively). There was no direct correlation between the *ras* oncogene and MMP gene expression.

To examine the effect of *ras* transfection on the expression of the MMPs, we used HT1080 fibrosarcoma which showed intrinsic expression of both MMP-2 and -9 mRNAs. The mRNAs for MMP-2, -9, and *ras* were analyzed in the cells which were transfected with the v-H-*ras* gene. The *ras* expression was increased in transfectants compared with the nontransfectant cells (data not shown). As shown in Fig. 2, the MMP-9 expression was significantly enhanced in the *ras*-transfected HT 1080 cells compared with the nontransfectants while the *ras* transfection did not affect the expression of MMP-2. This is consistent with the previous finding of Sato *et al.* (1992) on the enhanced expression of mRNA for MMP-9, but not MMP-2 in c-H-*ras*-transformed KMS -6 fibroblasts. The correlation between transformation by *ras* and enhanced expression of MMP appears to be cell type-specific since H-*ras*-transformed bronchial epithelial cells secreted MMP-2 which was not found in the normal parental cells (Collier *et al.*, 1988). Our results suggest the possible inducing effect of the *ras* oncogene on metastasis by activation of the MMP-9 gene in HT1080 cell line, though we do not provide a direct evidence of it. At present, work on the determination of MMP -9 enzymatic activity in *ras*-overexpressed HT1080 cells is being carried out. Moreover, whether or not the enhanced expression of MMP-9 by *ras* transfection accompanies the increased invasiveness of the cell remains to be determined.

Acknowledgement

We thank Dr. Kyu-Won Kim for providing the HT1080 cells and the DNA probes and for helpful discussion. Part of this work was carried out at the National Institute of Safety Research, Korea. This work was supported by the grants from the Ministry of Science and Technology and from Duksung Women's University, Korea.

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