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GELATINASES OF MURINE METASTATIC TUMOR CELLS

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SUMMARY: The properties of gelatinases secreted in culture medium of murine fibroblasts, macrophages, colonic carcinoma, FBJ virus-induced osteosarcoma, Lewis lung carcinoma and mammary tumor cells were compared. Normal fibroblasts and macrophages secreted gelatinases of 60,000 and 95,000 molecular weights, respectively. Tumor cells secreted both of these gelatinases, although the relative amounts of the 60 kDa and 95 kDa gelatinases differed among the cell lines. The cell lines that had the greatest metastatic potential to the lung secreted the highest amount of 95 kDa gelatinase. The 95 kDa gelatinase produced by tumor cells had properties similar to that of macrophages.

Enzymes that can degrade the components of the extracellular matrix have been reported to play an important role in tumor invasion and metastasis (1,2). In tumor cells with high metastatic potential, type IV collagen degrading enzymes are secreted and appear to participate in the process of tumor penetration into basement membrane (3,4). We have recently reported the detection of gelatinases with molecular weights of 95 kDa and 60 kDa in the conditioned medium of colonic carcinoma cells. Cell lines, clonally derived from these cancer cells, which secreted the most abundant amounts of gelatinase also had the highest metastatic potential (5). In the present study, we compared the gelatinase secretion of normal cells and various tumor cells with different metastatic capability. The results suggest a more wide-spread correlation between the secretion of a 95 kDa gelatinase and increased metastatic potential of tumor cells.

MATERIALS AND METHODS

Reagents: Aminophenylmercuric acetate and tunicamycin were purchased from Katayama Chemicals (Osaka) and Calbiochem-Behring Corp. (La Jolla, Calif.), respectively. RCA-agarose was the

Abbreviations: RCA, Ricinus communis agglutinin; Con A, concanavalin A; SDS, sodium laurylsulfate.

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product of Seikagaku Kogyo (Tokyo). The reagents for cell culture were the same as described previously (5). Other reagents were the purest grade available from commercial sources.

Cell Lines: Murine colonic carcinoma (Colon 26) cell lines of LuM1 and NM11 with high and low metastatic potentials to the lung, respectively, were described previously (5,6). FBJ virus-induced osteosarcoma cell line (FBJ-S1) and its metastatic variant (FBJ-LL) were obtained from FBJ virus-induced osteosarcoma of Balb/c mouse (7). Non-metastatic cell line, MuMT73-S6, was a cell line cloned by Dr. S. Saga from MuMT73 cells of spontaneous mammary tumors of Balb/cfC3H mice (8). A cell line of Lewis lung carcinoma (LL-3L) metastatic to the lung was obtained from lung metastatic tumors of C57BL/6 mouse subcutaneously transplanted by Lewis lung carcinoma (9). Details of these tumor cell lines will be published elsewhere. Macrophages were collected from peritoneal fluid of 6-8 week old female Balb/c mouse 4 days after the treatment of the mouse with thioglycolate according to Werb and Gorden (10), and inoculated into culture dishes. After 1 h, non-adherent cells were removed by vigorous washing with the culture medium. A cell line of Balb/c 3T3 fibroblasts was provided by Dr. M. Takahashi. All of these cells were cultured in RPMI 1640 medium with 10% fetal calf serum under 5% CO_2 in air at 37°C.

Measurement of gelatinase activity: Gelatinase activity was determined by zymography according to Chin et al (11) as described previously (5). In zymography, 10% polyacrylamide gel of 1 mm thickness was used, and the proteins were separated by Laemmli's buffer system (12). Cells (2.5×10^5) were inoculated into Corning culture dish (60 mm in diameter) with 3 ml culture medium. After overnight culture, medium was changed to HB101 or RPMI 1640 without fetal calf serum. The conditioned, serum-free medium obtained after 24 h culture was used for the measurement of gelatinase activity. In order to examine the effect of tunicamycin on gelatinase secretion, the cells were cultured first in the complete medium with tunicamycin (10 μg/ml) for 30 min, washed with the RPMI 1640 medium without serum, and then cultured in HB101 with tunicamycin. The conditioned medium after 17 h was tested for gelatinase activity.

Separation of gelatinases by RCA-agarose column: The same procedures as described previously were applied to separate 95 kDa gelatinases and 60 kDa gelatinases from culture media by RCA-agarose column chromatography (5). Gelatinase adsorbed was eluted from the column with 0.3M galactose.

Treatment of gelatinases with aminophenylmercuric acetate: To activate the latent form of gelatinases, gelatinase solutions were incubated with 1 mM aminophenylmercuric acetate for 4 h at 35°C.

RESULTS

Gelatin embedded gels were used to detect the activity and molecular weight of gelatinases secreted into the medium of different cell types grown in culture (Fig. 1). Mouse 3T3 fibroblasts and macrophages secreted 60 kDa and 95 kDa gelatinases, respectively (Fig. 1, lanes 1 and 8). The 60 kDa gelatinase was not detected in macrophage conditioned medium; nor was the 95 kDa gelatinase detected in fibroblast conditioned medium. All tumor cells secreted both 60 kDa and 95 kDa gelatinases except the non-

metastatic mammary tumor cells (Fig. 1, lane 4) in which only a 60 kDa gelatinase was observed. FBJ virus-induced osteosarcoma cells and colon 26 cells, which are highly metastatic cells, secreted increased amounts of 95 kDa gelatinases (FBJ-LL, Fig. 1, lane 3 and LuM1, Fig. 1, lane 6) compared to their low metastatic counterparts (FBJ-S1, Fig. 1, lane 2 and NM11, Fig. 1, lane 5). The conditioned medium of metastatic Lewis lung carcinoma cells contained the most striking amounts of a secreted 95 kDa gelatinase (Fig. 1, lane 7).

To determine whether one of the two observed gelatinases represented a precursor form of the other, the presence of asparagine-linked oligosaccharide side chains and the response of the two gelatinases to activation with aminopheylmercuric acetate was investigated. The 95 kDa gelatinases from macrophages and tumor cells were adsorbed to RCA-agarose column and eluted from the column by the competitive inhibitory sugar galactose (Fig. 2). The 60 kDa gelatinases from tumor cells did not react with the column. In the presence of tunicamycin in the culture medium, macrophages secreted a 88 kDa gelatinase and tumor cells secreted both 88 kDa and 60 kDa gelatinases (Fig. 3). These data taken

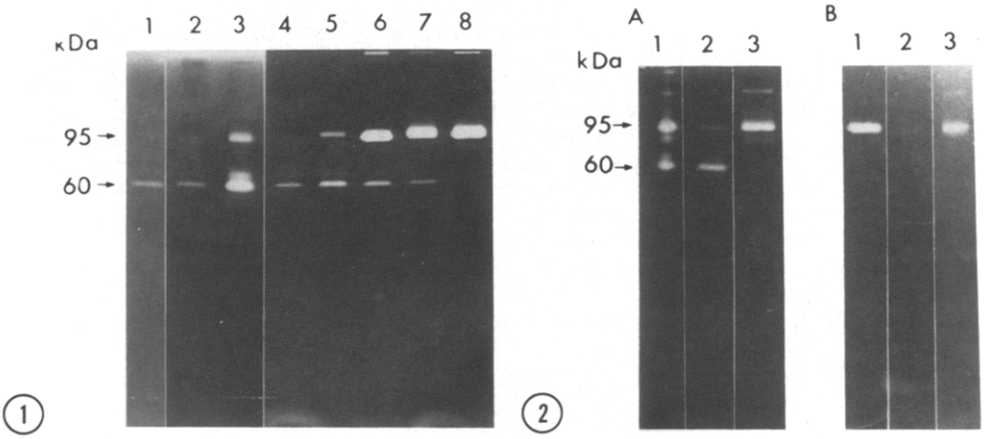
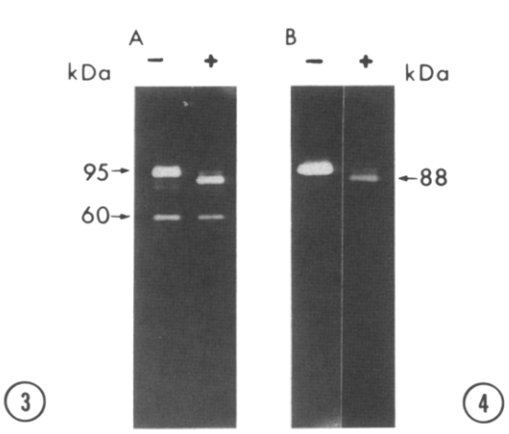


Figure 1. Gelatinases in serum-free, conditioned medium (RPMI 1640) of murine normal and tumor cells as detected by zymography with gelatin as a substrate. 7.5 µl of the medium of 24 h culture was submitted to SDS-gelatin-polyacrylamide gel electrophoresis and gelatinase activity was tested. Cells used were: lane 1, 3T3 fibroblasts; lane 2, FBJ-S1; lane 3, FBJ-LL; lane 4, MuMT73-S6; lane 5, NM11; lane 6, LuM1; lane 7, LL-3L; lane 8, macrophages.

Figure 2. Reactivity of gelatinases to RCA-agarose. The conditioned medium (100 µl) from LuM1 (A) or macrophages (B) was applied to RCA-agarose column (1 x 3 cm): lane 1, conditioned medium; lane 2, a fraction not adsorbed to the column; and lane 3, a fraction that eluted from the column.



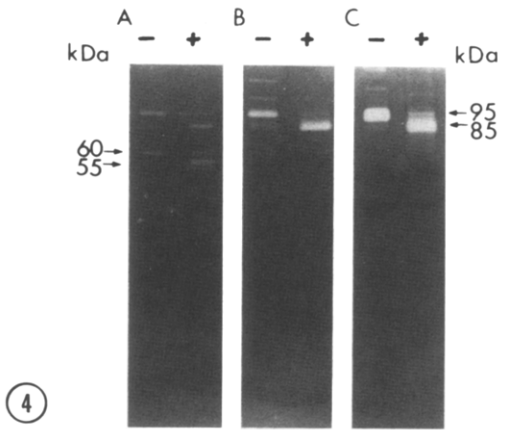


Figure 3. Gelatinase secretion in the presence of tunicamycin. 7.5 µl of serum-free, conditioned medium of LuM1 (A) and macrophages (B) prepared in the presence (+) or absence (-) of tunicamycin (10 µg/ml) was tested for gelatinase activities.

Figure 4. Effect of the treatment with aminophenylmercuric acetate on the molecular weight of gelatinases. Gelatinase activity was tested before (-) and after (+) the incubation of the gelatinase solution with 1 mM aminophenylmercuric acetate for 4 h at 35°C. A, gelatinase fraction of LuM1 not adsorbed to RCA-agarose; B, gelatinase fraction of LuM1 eluted from RCA-agarose; C, conditioned medium of macrophages.

together suggest that the 95 kDa gelatinases but not the 60 kDa gelatinases contain asparagine-linked oligosaccharide side chains. Treatment of the gelatinase solution with aminophenylmercuric acetate induced molecular weight reduction of both gelatinases: the 95 kDa gelatinases of tumor cells and macrophages to 85 kDa, and the 60 kDa gelatinases of tumor cells to 55 kDa (Fig. 4). Conversion of the 95 kDa gelatinases to the 60 kDa gelatinases was not observed in any of these experiments.

DISCUSSION

Gelatinases in the conditioned, serum-free medium of mouse cultured cells were detected by zymography with gelatin as a substrate. This method, in which proteins were first separated by SDS-polyacrylamide gel electrophoresis, provided both an estimation of the molecular weight as well as the amount of enzyme activity associated with each gelatinase. Although the amount of gelatinase activity differed from cell type to cell type, normal and tumor cells of the mouse secreted two gelatinases of 60 kDa and 95 kDa. Both gelatinases were metalloproteases activated by calcium ion and neither hydrolyzed bovine serum albumin and casein (data not shown).

Our results indicated that the 95 kDa gelatinases of macrophages and tumor cells had different properties from the 60 kDa gelatinases of fibroblasts and tumor cells. The 95 kDa gelatinases, but not the 60 kDa gelatinases, reacted to RCA-agarose (Fig. 2) and Con A-agarose (data not shown). After culture in the presence of tunicamycin, macrophages and tumor cells secreted 88 kDa gelatinases. These data, together with the results of reactivity to lectins, indicated that the 95 kDa gelatinases from macrophages and tumor cells were glycoproteins with asparagine-linked sugars and that the 88 kDa component produced in the presence of tunicamycin in the culture medium could be a polypeptide backbone of the 95 kDa gelatinase without asparagine-linked sugar side chains. The presence of tunicamycin did not affect the molecular weight of the 60 kDa gelatinases of tumor cells and fibroblasts suggesting that the 60 kDa gelatinases did not have asparagine-linked sugars. Treatment of the enzymes with aminophenylmercuric acetate to activate latent forms of metalloproteases, also induced changes of molecular weights of the gelatinases; the 95 kDa gelatinases decreased to 85 kDa and the 60 kDa gelatinases decreased to 55 kDa. Conversion of a 95 kDa gelatinase to a 60 kDa gelatinase has not been observed. These results suggested that there were at least two gelatinases in the mouse cells differing in their molecular weight and glycosylation.

The presence of gelatinases of different molecular weights has been observed in other mammals as well. For example, human macrophages have been reported to secrete a 90 kDa gelatinase (13) and human fibroblasts have been shown to have a 67 kDa gelatinase. An antibody to neutrophil gelatinase has been reported to cross-react with the gelatinase from macrophages but not with that from fibroblasts (13,14). Thus, in human cells, at least two gelatinases are present differing in their molecular weights and immunochemical properties.

In tumor cells, the pattern of gelatinase secretion differed among the cell lines. A non-metastatic cell line (MuMT73-S6) secreted only a 60 kDa gelatinase and other tumor cells secreted both a 95 kDa and a 60 kDa gelatinase. When compared with low metastatic cell lines, secretion of 95 kDa gelatinases markedly increased in the conditioned medium of metastatic cell lines of FBJ virus-induced osteosarcoma and colonic carcinoma cells.

Metastatic Lewis lung carcinoma cells also secreted large amount of a 95 kDa gelatinase. These results suggested that enhancement of secretion of 95 kDa gelatinases had some correlation with the spontaneous metastatic property of a given tumor cell line. The induction of a gelatinase with similar molecular weight and its correlation with the malignant phenotype of the cells were recently reported in the culture supernatant of NIH/3T3 transfected by the ras oncogene (15).

The 95 kDa gelatinases secreted by tumor cells had characteristics similar to the 95 kDa gelatinase of macrophage with respect to reactivity to lectins, effects of tunicamycin and activation by aminophenylmercuric acetate. In response to pathophysiological conditions of the host, macrophages have been known to migrate in host tissues, and it has also been reported that secretion of neutral proteases in macrophages was markedly enhanced by the stimulation of macrophages (10). Our present finding that tumor cells with a metastatic potential to the lung secreted a gelatinase that had properties similar to macrophage gelatinase suggested the correlation of 95 kDa gelatinase production and metastatic capability of cells. Activated secretion of a 95 kDa gelatinase could be a significant attribute of metastatic cells and play an important role in their migration through the extracellular matrix.

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