Letter to the Editor

SELF-REGULATION OF TYPE I COLLAGEN DEGRADATION BY COLLAGEN-INDUCED PRODUCTION OF MATRIX METALLOPROTEINASE-1 ON CHOLANGIOCARCINOMA AND HEPATOCELLULAR CARCINOMA CELLS

Dear Editor:

Malignant tumors are often invasive and result in metastatic foci, and the metastases restrict the method of treatment. If the mechanism of metastasis of malignant cells were known, it might be possible to develop new approaches to therapy. Much research has been performed to elucidate this mechanism in many kind of carcinoma. As the first step of metastasis, it is important for cancer cells to detach themselves from original foci and to break surrounding matrices. Matrix metalloproteinase-1 (MMP-1) is known as a principal enzyme for the breakdown of fibrillar collagens and is considered an important factor in the first step of metastasis. It is a zinc-dependent protease that is secreted as proform and becomes activated through proteolytic cleavage. On the other hand, a tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) inhibits MMP-1 activity through non-covalent binding of the active form of MMP-1 at molar equivalence. Recently, Vogel et al. (1997) clarified in vitro on a kind of fibrosarcoma cells, HT 1080, into which a discoidin domain receptor 2 (DDR2) expression vector was introduced, that fibrillar collagens are the ligands of DDR2, a receptor of tyrosine kinase, which was first reported as an orphan receptor. Furthermore, they found that the activated DDR2, by linking to the collagens, promoted the production and secretion of MMP-1. Fibrillar collagens have also been known as the ligands of integrins α1β1 and αVβ3, and Riikonen et al. (1995) reported on osteogenic cell lines that integrin α1β1 upregulated the expression of MMP-1 after stimulation by type I collagen. If these findings can be applied to all kinds of cells, they would offer a useful clue for the development of new protective methods against metastasis. We have prepared cholangiocarcinoma (CC) and hepatocellular carcinoma (HCC) cell lines of various stages of differentiation with metastatic activity. Here we ascertained the expression level of cell surface DDR2 and integrin α1β1 on these cell lines and attempted to determine how the cell-collagen interaction influences the production of MMP-1 and TIMP-1.

Seven HCC and CC cell lines were used for this study. The RBE and SSP-25 cell lines were derived from the same CC nodule in the liver of a patient with metastatic foci in the lung (Enjoji et al., 1997a). The RBE was from adenomatous cells, and the SSP-25 was from sarcomatous cells, because the phenotypes of RBE and SSP-25 were completely the same as those of adenomatous and sarcomatous tumor cells in tissue sections, respectively. Morphologically, SSP-25 cells were spindle shaped, like the sarcomatoid cells in the CC nodules. The ETK-1 cell line is thought to have invasive activity because it was established from ascitic fluid of a patient with CC (Enjoji et al., 1997b). The ETK-1 cells are small polygonal cells and have bipotential liver stem cell-like characteristics. The ETK-1 cells could differentiate along hepatocytic lineage after 5-azacytidine treatment, and then the differentiated HCC cells were cloned as MEK cell line. When the ETK-1 cells were transplanted to mice subcutaneously, tubular CC was organized with mature phenotypic characteristics, and after cloning the resultant clones were designated as NEC cell line. These five cell lines, RBE, SSP-25, ETK-1, MEK, and NEC, were used for the following experiment. Other well-known HCC cell lines, HepG2 and HLE, were also studied.

Cell surface expression of DDR2 was analyzed by flow cytometry. Additionally, the presence DDR1 expression was determined. Activation of DDR1 is triggered by collagen types I, II, III, IV, and V, and DDR2 is activated only by collagen types I and III (Vogel et al., 1997). However, the percentage of DDR1-positive cells was under 5% in all analyzed cell lines (data not shown). A variety of DDR2 expression patterns were shown according as the cell type (Fig. 1). Both RBE and SSP-25 cells, which are from the same CC nodule but in various stages of differentiation, showed little DDR2 expression on their cell surface. The DDR2 expression on HepG2 was at a low level; ETK-1, MEK, and NEC, which are of the same origin, showed a moderate to high expression level. Highest expression level was with HLE. Cell surface expression of integrin α1β1 has been already reported (Enjoji et al., 1998). Integrin α1β1 exists amply on the surface of all cell lines used in this study with the exception of SSP-25. From these data, no correlation between the expression of these collagen receptors and stage of differentiation, metastatic activity, or cell lineage was found.

As mentioned previously, seven cell lines were assayed for MMP-1 and TIMP-1 secretion. First, all seven cell lines were stimulated by culturing on type I (fibrillar) collagen-coated dishes, a ligand for DDR2 and integrin α1β1, or membranous (type IV) collagen-coated dishes used for a negative control. However, there was no significant change in secreted MMP-1 and TIMP-1 in the media compared with control cells with no stimulation (data not shown). This finding is reasonable because it has been reported that the binding sites of type I collagen recognized by integrin α1β1 are conformation dependent and that thin layers of coating with type I collagen is not effective as a stimulator (Gullberg et al., 1992; Ellerbroek et al., 1999). Next, we adopted dissolved collagens as stimulators. Cells (2 × 10⁵/well) were cultivated in RPMI 1640 containing 10% FBS in six-well plates. After a 24-h incubation, media were changed to serum-free RPMI 1640 (1 ml/well) containing 10% FBS in six-well plates. After a 24-h incubation, media were changed to serum-free RPMI 1640 (1 ml/well) and cultured with or without dissolved human type I or type IV collagen (final concentration, 10 μg/ml) for 24 h. Then conditioned media were collected and analyzed for the presence of secreted MMP-1 and TIMP-1 by Western blotting with monoclonal anti-MMP-1 and anti-TIMP-1 antibodies (Fig. 2). Type IV collagen stimulation showed no effect for MMP-1...
Fig. 1. Flow cytometry analysis of the expression of DDR2 in ETK-1, MEK, NEC, HepG2, SSP-25, RBE, and HLE cells. Data are presented as histograms of cell number (y-axis) versus fluorescence intensity (log scale, x-axis). Negative controls are presented as thin lines.

Fig. 2. Western blotting for detecting MMP-1 and TIMP-1. Culture media of each cell line were collected after type I collagen stimulation (I), type IV collagen stimulation (IV), or no stimulation (−) and used as samples.

secretion. After type I collagen stimulation, the secreted MMP-1 level was significantly upregulated with the NEC, RBE, SSP-25, HepG2, and HLE cell lines by two- to sevenfold, while MMP-1 secretion from ETK-1 and MEK was not influenced by the stimulation. The TIMP-1 secretion was almost constant on all tested cell lines whether or not these collagens were added.

The collected media, which was 20-fold concentrated by using Microcon centrifugal filter devices (Millipore, Bedford, MA), were also used for collagenase assay by the Type I Collagenase Activity Assay Kit (Yagai, Yamagata, Japan). Briefly, after collagenase activation by trypsin, 50 μl of the concentrated media were incubated with 100 μl of fluorescein isothiocyanate–labeled type I collagen for 3 h at 37°C. The reaction was terminated by the addition of 80 mM o-phenanthroline. Type I collagenase (MMP-1) activity was estimated by measuring the fluorescent intensity of the supernatant at 495 nm (excitation)/520 nm (emission) by a fluorescence spectrophotometer. The ratio of secreted MMP-1 activity of type I collagen stimulated to unstimulated cells is shown in Fig. 3. The MMP-1 secretion increased after type I collagen stimulation except in ETK-1 and MEK cell lines. Naturally, the result coincided with that of Western blotting.

Here we evaluated whether type I collagen stimulation regulates the expression level of MMP-1 and its inhibitor, TIMP-1, and whether the amount of expression of DDR2 and integrin α2β1 correlates with responsiveness to the collagen. Our results suggest the tendency with CC and HCC cells that type I collagen degradation is self-regulated by collagen-induced MMP-1 production, although the phenomenon is not true for all cases. However, there was no correlation between MMP-1 secretion level after type I collagen stimulation and the cell surface expression level of DDR2 or integrin α2β1. In cases of ETK-1 and MEK, MMP-1 secretion was not...