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Potentiation of Invasive Capacity of Rat Ascites Hepatoma Cells by Transforming Growth Factor-β

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The *in vitro* invasive capacity of poorly invasive cells (W_1), which were cloned from rat ascites hepatoma cells (AH 130), was potentiated dose- and time-dependently by pretreating the cells with transforming growth factor-β (TGF-β). This potentiation of invasive capacity was completely abolished by anti-TGF-β antibody. When the treated cells were ip inoculated into rats, the cells extensively invaded the peritoneum, and formed penetrating tumor nodules. The effect of TGF-β was reversed by subculturing the treated cells without TGF-β. The potentiation of invasive capacity by TGF-β might participate in platelet-associated enhancement of tumor cell metastasis.

Key words: Invasion — Transforming growth factor-β — Mesothelial cell —Ascites hepatoma cell

Metastasis is a multistep phenomenon including the detachment of malignant cells from the primary tumor, intravasation, circulation of the free tumor cells, and their lodging at the target organs where they proliferate and form secondary tumor foci. Invasion appears to be the most characteristic step in metastasis. To understand the mechanisms by which the invasion takes place, we have developed a culture system for studying tumor invasion.[1] By using this model system, we recently found that the invasiveness of tumor cells is not only genetically determined but is greatly influenced by their interactions with host cells and mediators.[2–4] For instance, when AH 130 cells were precultured with macrophages (tumor cell/macrophage ratio >1) or pretreated with oxygen radicals generated in a medium containing hypoxanthine-xanthine oxidase (EC 1.2.3.2), both the *in vitro* and *in vivo* invasive capacities of the tumor cells were potentiated. A certain peptide extracted from rat liver strongly suppressed the invasion of AH 130 cells. Moreover, the preculture of murine melanoma cells with fibroblasts markedly enhanced the lung colonizing ability of the tumor cells.[5] In the present paper, we describe the induction by transforming growth factor-β (TGF-β) of the invasive capacity of a poorly invasive cell line (W_1) cloned from AH 130 cells.

AH 130 cells were maintained by serial ip transplantation into male Donryu rats. AH 130 cells from the peritoneal cavity were cultured in Eagle's minimum essential medium with 2-fold concentrations of amino acids and vitamins supplemented with 10% fetal calf serum in an atmosphere of 95% air and 5% CO_2 at 37°C. The poorly invasive clone, W_1, was isolated from the cultured parental AH 130 cells by limiting dilution. W_1 did not adhere to the culture dish and was maintained in suspension culture. The *in vitro* invasive capacity of the tumor cells was assayed by counting tumor cell colonies formed underneath the mesothelial cell (M-cell) monolayer as reported previously.[2] Briefly, M-cells were isolated from rat mesentery by trypsin digestion and cultured as a monolayer. When M-cells had grown almost to confluency, 2×10^5 AH 130 cells were seeded on the M-cell monolayer and cultured for 24 h. The number of penetrating tumor cell colonies that developed underneath the M-cell monolayer in 60 different visual fields (1.13 mm^2 each) was counted under a phase contrast microscope with the aid of a grid drawn on the lid of the culture dish and expressed as penetrating colonies/cm^2, which was defined as the *in vitro* invasive capacity.

When W_1 cells (2×10^5/dish) were pretreated with TGF-β (1.25–30 ng/ml, Wako Pure Chemical Industries, Ltd.), the cells adhered to and spread on the plastic culture dish in about 24 h, developing an epithelial cell appearance. The growth of W_1 in the presence of TGF-β was inhibited by 10–20% compared with that in the absence of TGF-β. The *in vitro* invasive capacity of the cells recovered from the dish by scraping with a rubber cleaner was measured (Fig. 1). A significant increase in the invasive capacity was observed from 1.25 ng/ml TGF-β and reached the maximum at levels of more than 10 ng/ml TGF-β (about 15 times more invasive than the untreated control). The potentiation by 10 ng/ml TGF-β depended on the time period of the pretreatment; the effect increased with time up to about 24 h (Fig. 2). As shown in Table I, the potentiation by TGF-β was completely inhibited by anti-TGF-β antibody (R&D systems, Inc.). The acquisition of adhesive character of the cells was also blocked by the addition of the antibody. When the TGF-β-treated cells were ip inoculated into rats, the

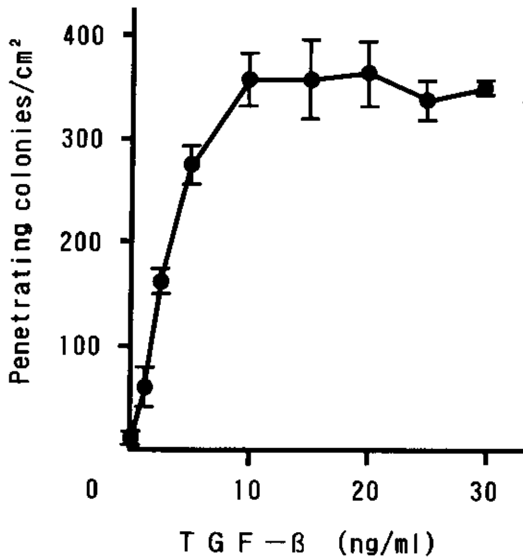

Fig. 1. Dose-dependent potentiation of the *in vitro* invasive capacity of W_1 by TGF-*β*. W_1 cells (2×10^5/dish) were pretreated for 24 h in the presence of various concentrations of TGF-*β*, washed, and tested for *in vitro* invasive capacity. Data points are mean values±SD.

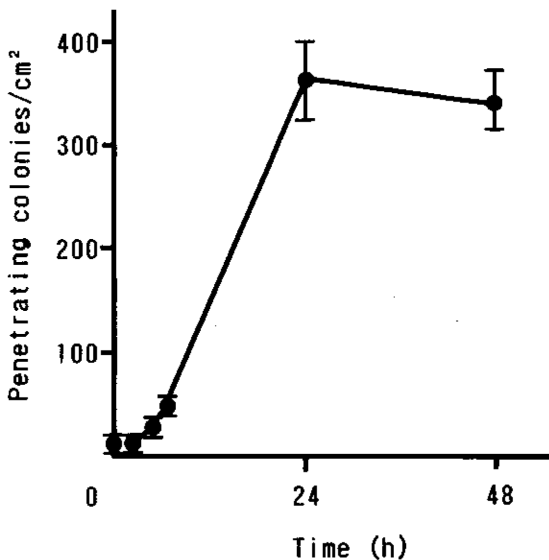

Fig. 2. Time-dependent potentiation of the invasive capacity of W_1 by TGF-*β*. W_1 cells (2×10^5/dish) were pretreated with TGF-*β* (10 ng/ml) for the time periods indicated, washed and tested for *in vitro* invasive capacity. Data points are mean values±SD.

Table I. Effect of Anti-TGF-*β* Antibody on the Potentiation of Invasive Capacity by TGF-*β*

W_1 pretreated with	Number of penetrating colonies/cm²
None	23±14[a]
TGF-*β*	360±50
TGF-*β*+anti-TGF-*β*	27±5

W_1 cells (2×10^5/dish) were pretreated with 10 ng/ml TGF-*β* in the presence and absence of anti-TGF-*β* antibody (35 μg/ml) for 24 h, then washed and assayed for *in vitro* invasive capacity.
a) Mean±SD from at least 3 determinations.

Fig. 3. *In vivo* invasion of W_1 pretreated with TGF-*β*. W_1 cells (8×10^6) that had been pretreated with and without 10 ng/ml TGF-*β* for 48 h were ip implanted into rats. Rats were sacrificed 12 days later. a, Tumor nodules (arrows) formed in the peritoneum of rats that had received W_1 pretreated with TGF-*β*; b, a macroscopic view of the visceral organs of a control rat that had received the untreated W_1.

cells extensively invaded the peritoneum and formed penetrating tumor nodules, whereas the inoculation of the untreated control failed to yield any detectable penetrating nodule (Fig. 3). On transferring the treated cells to and culturing them in a fresh culture medium without TGF-*β*, the elevated invasive capacity of the cells decreased sharply in 24 h (about 80% decline) and returned to the original low level in 6 days (Fig. 4). On the other hand, all the treated cells remained adherent during the 1st 24 h and thereafter started to detach from the culture dish surface. The detachment was completed by the 6th day. Whether or not the acquisition of the adhesive property of the cells correlates with their invasiveness is unclear, but the lag of the disappearance of the adhesive property suggests no direct correlation between them.

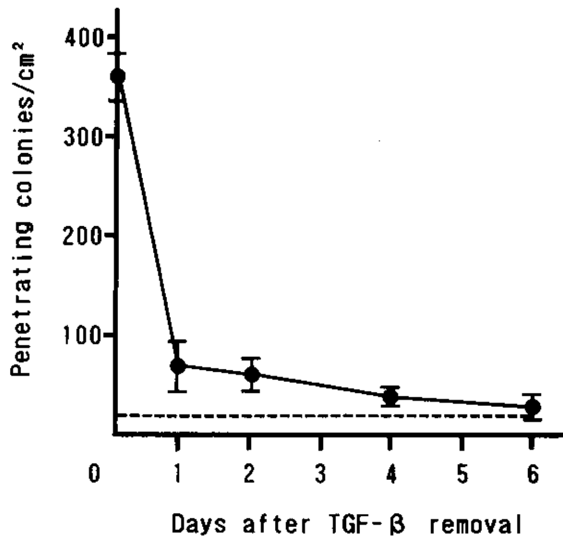

Fig. 4. Reversibility of the effect of TGF-β. W_1 cells (2×10^5/dish) were pretreated with TGF-β (10 ng/ml) for 24 h, washed, transferred to a fresh culture medium, and cultured without TGF-β. At intervals, the tumor cells were tested for their *in vitro* invasive capacities (●—●). -----, The invasive capacity of W_1 before the pretreatment. Data points are mean values±SD.

TGF-β is a multifunctional regulator of cellular growth and differentiation in many cells.[6,7] It has been found to stimulate fibrosis, angiogenesis and wound healing.[8,9] Platelets have been reported to contain a much larger quantity of TGF-β than other cells and tissues.[10] The platelet-aggregating ability of certain tumor cells has been reported to have a crucial role in the formation of metastases.[11-13] The present results suggest that tumor metastasis enhanced by the tumor cell-platelet aggregation series may result from the potentiation of the invasive capacity of the tumor cells by TGF-β which is released from the activated platelets. In fact, a preculture of W_1 with thrombin- or collagen-activated platelets was found to potentiate the invasive capacity of W_1, and this potentiation was suppressed by the addition of anti-TGF-β antibody (unpublished results). The mechanism by which TGF-β enhances the invasive capacity is not known at present. Since TGF-β has been reported to stimulate collagen and fibronectin production,[8,14] the potentiation of invasive capacity by TGF-β might be mediated by the overproduced collagen and fibronectin. However, that is unlikely because collagen gel located under the M-cell monolayer did not enhance the invasive capacity of W_1, and a pentapeptide (GRGDS), the cell-binding domain of the fibronectin molecule, did not inhibit the invasive capacity of the tumor cells (unpublished results).

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