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0014-4754/87/060611-04$1.50 + 0.20/0
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Enhancement of fibronectin expression by herbimycin A[1]

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Summary. Herbimycin A specifically increased the level of fibronectin mRNA in Rous sarcoma virus-infected rat kidney cells, and the time course of fibronectin expression was found to be closely related to that of morphological change induced by herbimycin A.
Key words. Herbimycin A; fibronectin; β-actin; rat kidney cells.

Herbimycin A was isolated from the culture filtrate of *Streptomyces* in 1979 as an ansamycin antibiotic with herbicidal activity[2]. Recently, it was again isolated from the culture broth of *Streptomyces* sp. MH237-CF8 as an active substance that causes reversion of the transformed morphology of Rous sarcoma virus-infected rat kidney cells to the normal morphology[3]: herbimycin A-treated cells infected with a temperature-sensitive mutant of Rous sarcoma virus became flattened and more elongated and showed similar morphology to that of cells at the non-permissive temperature.

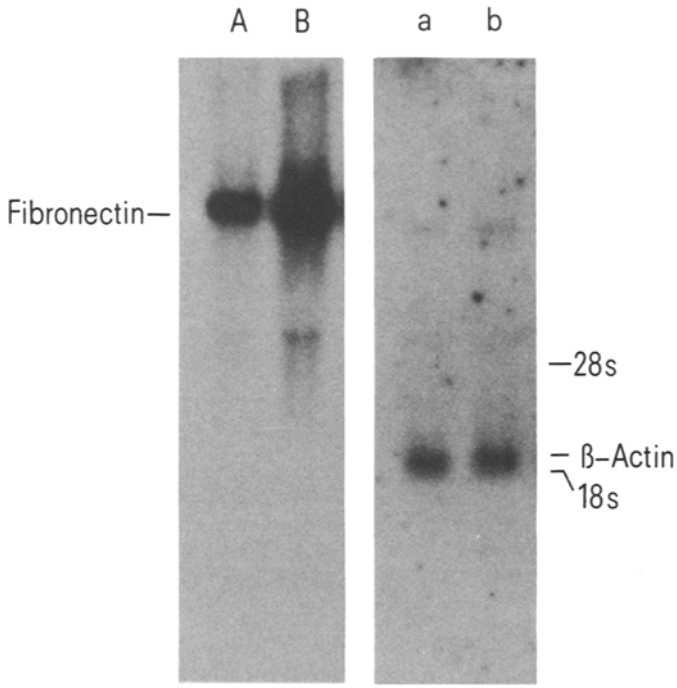

Figure 1. Fibronectin expression in ts/NRK cells. The cells were incubated at 33 °C (*A*) or 39 °C (*B*) for 2 days and harvested for RNA extraction. 10 μg of total RNA was applied on agarose gel electrophoresis and after Northern blotting, the blotted filter was hybridized with fibronectin cDNA (*A* and *B*) or β-actin gene (*a* and *b*).

Fibronectin is a large glycoprotein that plays an essential role in cell-cell and cell-basal lamina adhesion[4]. It is located on the cell surface and in plasma, and its primary structure has recently been elucidated[5]. Transformed cells have a reduced level of fibronectin on their surface and this decrease is known to be regulated at the transcriptional level[6]. Therefore, we have studied the effect of herbimycin A on fibronectin expression in tumor cells.

Materials and methods. Herbimycin A was kindly supplied by Dr Y. Uehara, National Institute of Health, Tokyo. A human fibronectin cDNA clone, pFH1[7], was a gift from Dr A.R. Kornblihtt, INGEBI, Buenos Ayres, and the β-actin gene, pRβAc3'ut[8], was kindly supplied by Dr K. Tokunaga, Chiba Cancer Center Research Institute. Normal rat kidney cells infected with the temperature-sensitive mutant of Rous sarcoma virus (ts/NRK cells)[9] were obtained from Dr M. Yoshida, Cancer Institute, Tokyo. The cells were grown at 33 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin G and streptomycin. Approximately 2×10^6 cells were seeded into 40 ml of medium in a Nunc 175-cm^2 flask and harvested in the sub-confluent state after culture for 54 h. Herbimycin A was added for the indicated times.

Total RNA was extracted from sub-confluent monolayers by the guanidine hydrochloride method[10]. About 300 μg of RNA was extracted from cells in 5 Nunc 175-cm^2 flasks. Transfer of RNA from electrophoresis gels containing formaldehyde to nitrocellulose membranes and hybridization to the pFH1 probe were carried out as described in the manual of T. Maniatis et al.[11].

Results and discussion. ts/NRK cells show the morphology of tumor cells with criss-crossing and piling up at the permissive temperature (33 °C) and flattened normal morphology with contact inhibition at the non-permissive temperature (39 °C)[9]. As shown in figure 1, expression of fibronectin was markedly in-

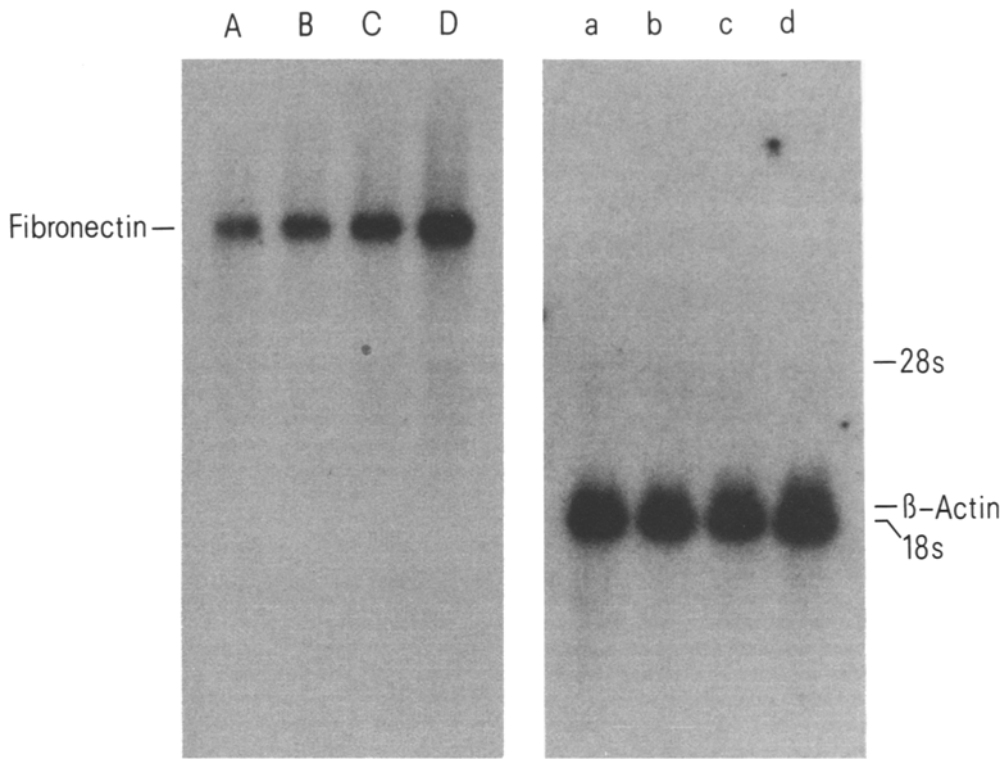

Figure 2. Enhancement of fibronectin expression by herbimycin A. The ts/NRK cells were incubated at 33 °C without herbimycin A (*A*) or with 0.5 μg/ml herbimycin A for 6 h (*B*), 12 h (*C*) or 30 h (*D*) and harvested for RNA extraction. Each RNA was run on a gel and hybridized as described in the legend of figure 1. *A–D* Fibronectin expression; *a–d* β-actin expression.

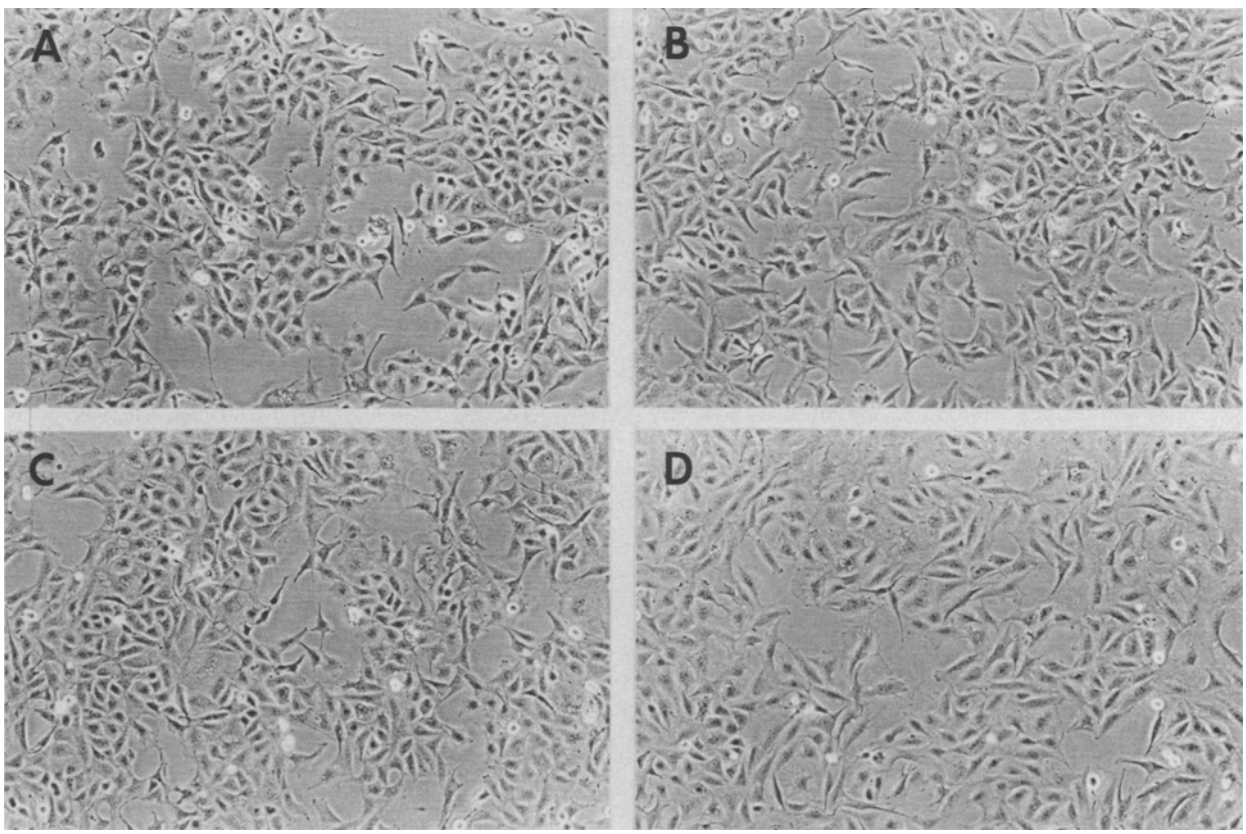

Figure 3. Time course of morphological effect of herbimycin A on ts/NRK cells. the cells were incubated at 33 °C without herbimycin A (*A*) or with 0.5 μg/ml herbimycin A for 6 h (*B*), 12 h (*C*) or 30 h (*D*), and photographed under the phase-contrast microscope.

creased when the cells were incubated at the non-permissive temperature, while expression of β-actin did not change.

Addition of 0.5 µg/ml of herbimycin A for 6–30 h to cultures of ts/NRK cells at the permissive temperature increased fibronectin expression and also changed the cell morphology, as shown in figure 2 and figure 3, respectively.

Addition of herbimycin A for 30 h enhanced fibronectin expression to almost the same level as that in normal cells, and altered the cell morphology to that of normal cells, as shown in figure 2D and figure 3D, respectively. Addition of herbimycin A did not change the expression of β-actin, as shown in figure 2a–d. Thus, herbimycin A specifically increased the level of fibronectin mRNA and the time course of this increase was closely related to the morphological change. We also found that enhancement of fibronectin expression by herbimycin A was reversible, as was the morphological change.

Cell surface fibronectin is linked to intracellular microfilaments and plays a role in construction of the cytoskeleton and in cell morphology[12]. Addition of fibronectin to the tumor cells is known to change their cell shape to that of normal cells[13]. Therefore, it is likely that induction of morphological change by herbimycin A was due to its enhancement of fibronectin synthesis.

1 Acknowledgments. The authors wish to thank Dr Y. Uehara, National Institute of Health, Tokyo, and Dr K. Hayashi, National Cancer Center Research Institute, Tokyo, for valuable suggestions. This work was supported in part by grants from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan.

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0014-4754/87/060614-03$1.50 + 0.20/0
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Relationships of the soluble human A, B, and H antigens of blood group A_1B individuals

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Summary. Ouchterlony double diffusion reactions with precipitating antibodies and lectins provide visual evidence that in saliva of A_1B secretor individuals the A and B antigens are predominantly situated on the same molecule, while the H antigen is on a different molecule.

Key words. Human ABH antigens; soluble substance; precipitin reaction; lectins; antibodies.

The human A, B, and H antigens are not only present on red cells and most tissue cells, but in a soluble form they may be detected in serum, and according to a defined pattern (secretor, nonsecretor), may also be present in other body secretions including saliva. The double diffusion precipitin reaction has rarely been used in the study of the soluble A and B antigens, presumably because the human anti-A and anti-B are non-precipitating antibodies. Yet, the double diffusion precipitin reaction is a powerful technique for determining similarities and differences between antigens and between antibodies and lectins. Findings on double diffusion precipitin reactions against the soluble A, B, and H antigens of A_1B saliva are presented in this report.

Saliva samples were obtained from individuals of a previously described study[1]. Unstimulated saliva samples were collected throughout the study. Specific anti-A antibodies were produced in rabbits as previously described[2]. Selected rabbits which lacked the A antigen in their saliva were repeatedly injected with human group A red cells. The immune sera were inactivated, diluted with an equal amount of saline, and absorbed with pooled human group B as well as O cells. Five or more absorptions, each with equal volumes of washed packed erythrocytes, were required to remove the detectable traces of anti-B and nonspecific antihuman reactivity. The anti-A reagents were cleared by twice-repeated precipitation of the γ-globulin fraction with saturated ammonium-sulfate in a ratio of 2 parts of ammonium-sulfate to 3 parts of reagent. The final precipitate was re-dissolved in phosphate buffered saline, and the ammonium-sulfate was removed by dialysis against saline at 4 °C.

Attempts to produce anti-B in rabbits yielded unsatisfactory results, presumably because all rabbits possess a B-like antigen. However, goats produced an anti-B of high titer suitable for precipitin reactions. These antisera were purified by selective absorption with group O and A red cells.

The lectins of *Ulex europaeus* were purified by fractionation with increasing concentrations of ammonium sulfate, followed by column chromatography separation. At relatively low concentrations of ammonium sulfate (e.g. 40%) the precipitate was rich in L-fucose specific anti-H. These fractions were purified further with a DEAE Fractogel column followed by an L-fucose affinity column to obtain the Ulex I fraction[3].

Double diffusion precipitin reactions were carried out in agar plates as previously described[4]. However, in this study, 0.5% SeaKem HGT(P) agarose (FMC Corporation, 5 Maple Street, Rockland, Maine 04841) was substituted for agar-Noble. The agar plates were incubated at 4 °C in a moist box and inspected daily for precipitin bands. Optimal band formation was observed after 2–5 days. Photographs were taken against a dark background with indirect light from 4 high intensity bulbs at an angle below the agar plate.