Acta Derm Venereol (Stockh) 1990; 70: 185–188

Contraction Phenomenon of Type I Collagen Gel by Melanoma Cells

TAKESHI KONO, MASAYOSHI FURUKAWA, TSUKASA TANII, JUN-ICHI KITAJIMA, NOBUYUKI MIZUNO, MASAMITSU ISHII and TOSHIO HAMADA

Department of Dermatology, Osaka City University Medical School, Osaka, Japan

In order to study the interaction between melanoma cells and collagen, B16 murine melanoma cells were embedded and cultured in type I collagen gel. Melanoma cells cultured in the collagen gel became elongated, as compared with those cultured on plastic, and some of them assumed a dendritic form. The gel contracted very slowly but steadily during culturing of melanoma cells, as in the experiment using fibroblasts. On the 20th day of culture the area of the gel accounted for only 32% of that when culture started. This contraction was enhanced by retinoic acid, which is known to induce cell differentiation. The contractility of the gel differed between various lines of melanoma cells. The present observations raise the possibility of interaction between melanoma cells and type I collagen.

(Accepted August 30, 1989.)

Acta Derm Venereol (Stockh) 1990; 70: 185–188.

T. Kono, Department of Dermatology, Osaka City University Medical School, 1-5-7, Asahimachi, Abeno-ku, Osaka 545, Japan.

Melanoma is one of the malignancies that are potentially highly metastatic. Unfortunately, much still remains to be elucidated about the mechanism of metastasis. In the first step of metastasis, melanoma cells infiltrate the dermis and inevitably encounter type I collagen, the largest component of connective tissue. They then migrate and proliferate, surrounding the collagen. Several recent reports have described a correlation between metastatic potential and enzyme activity related to the collagen metabolism (1, 2), and a collagen-induced alteration of glycosaminoglycan (GAG) synthesis in melanoma cells (2, 3), both of which are suggestive of interaction between melanoma cells and collagen.

By contrast, Bell et al. (4) reported the very peculiar phenomenon that the collagen gel in which fibroblasts were cultured underwent rapid contraction. Although the mechanism of this gel contraction has not yet been fully elucidated (5), the culture system using collagen gel is useful for the study of interaction between cells and collagen. In this work we cultured B16 melanoma cells and certain other cell lines in type I collagen gels and evaluated gel contractility.

MATERIALS AND METHODS

Cell culture

A B16 melanoma clone (B16/CBC) was derived from a subcutaneous tumour, resulting from the transplanatation of B16 C_2M melanoma cells (6) (kindly donated by Dr A. Oikawa, Tohoku University, Sendai, Japan) into a C57BL mouse and cloned with limiting dilution. The B16/CBC line was maintained in Eagle's medium (Nissui Pharmaceutical Co., Tokyo, Japan) with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY).

The human melanoma cell line AKI (7) was generously donated by Dr A. Ishii (Toranomon Hospital, Tokyo, Japan), and the SEKI line was kindly presented by Drs H. Okumura and K. Yamada (National Institute of Health, Tokyo, Japan). A Syrian hamster melanoma cell line (RPMI 1846) was purchased from the American Type Culture Collection (Rockville, Md.). These three lines were all maintained in RPMI 1640 medium (Nissui Co.).

Preparation of collagen gel

Prior to use, the cells were briefly treated with a 0.25% trypsin (GIBCO) solution, washed twice, counted, and diluted with medium to the desired concentrations.

The melanoma cells were embedded three-dimensionally in hydrated collagen gels using a modified version of the procedure developed by Elsdale & Bard (8). The following stock solutions were prepared and kept below 4°C. Solution I: 0.3% (w/v) pepsinized type I collagen solution in dilute HCl (pH 3.0) (derived from porcine tendon and checked for purity by SDS–PAGE, Nitta Gelatine Co., Osaka, Japan); Solution II: Eagle's medium concentrated ten-fold; and Solution III: 200 mM HEPES in 0.08 N NaOH. Eight volumes of Solution I and one volume of Solution II were mixed in a plastic tube with one volume each of Solution III and FCS (collagen mixture). After the cell suspension containing the appropriate number of cells (1 : 10 volume of total collagen mixture) was added to the tube, 1 ml of this mixture was poured into a 35-mm bacteriological dish (Falcon). The dishes were incubated at 37°C. The collagen gelated within 10 min and the cells were trapped three-dimensionally within the gels. The final collagen concentration was 2.0 mg/ml. Initially 10^6 cells were incorporated in the 1 ml of collagen mixture.

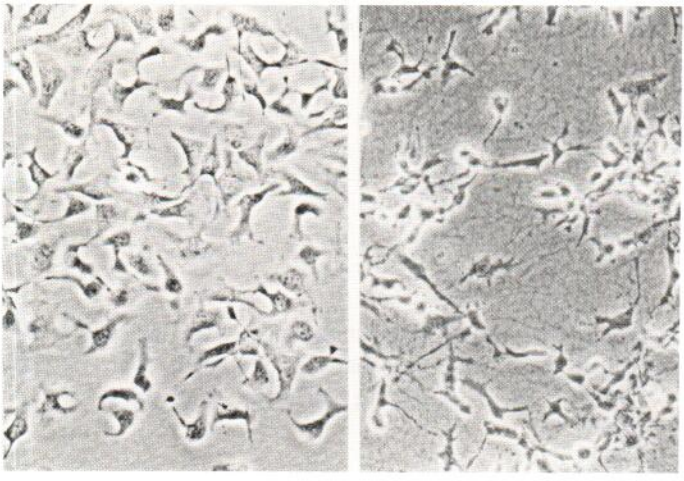

Fig. 1. Phase-contrast image of B16 melanoma cells cultured on the ordinal plastic (*left*) and within the collagen gel (*right*). Fifth day of culture. ×250.

Measurement of gel size

The diameter of each gel was measured every 24 h by placing the dishes on a ruler and the means of diameters in three planes were recorded.

Measurement of cell viability

At 20 days of culture, the gels were minced with scissors and digested with collagenase (4 mg/ml, Sigma, Type IV) at 37°C for 40 min. The released cells were collected by centrifugation at 1,000 rpm for 5 min. The cells were resuspended in a 0.04% trypan blue solution in phosphate-buffered saline. The viable cells were counted with a hemocytometer and the viability (percentage of dye-excluded cells) was determined.

RESULTS

Fig. 1 shows the morphological findings after culturing B16 melanoma cells on plastic and in collagen gel. Those cultured on plastic were flattened and the individual processes were broadened (Fig. 1, left). In contrast, those cultured in the gel were elongated with fine processes, and some were dendritic (Fig. 1, right).

Gel contraction occurred gradually during long-term culture of B16 melanoma cells, and the gel floated on the culture medium. Fig. 2 shows the contracted gel on the 10th day of culture. The gel was darkened due to melanin deposition.

Fig. 3 displays the time-course changes in the diameter of collagen gel. The gel containing 10^6 cells contracted by degrees and its area decreased to 32% of the original area (day 0) of culture.

When culturing was performed in the presence of retinoic acid (RA), which is known to induce differen-

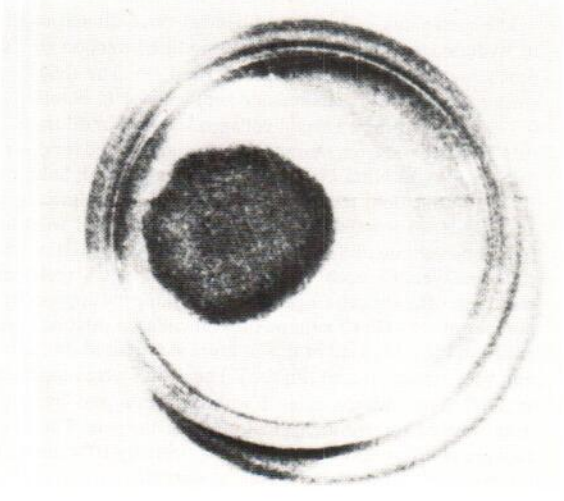

Fig. 2. Contracted collagen gel was floated in the medium. Melanoma cells (10^6 cells) were incorporated in the collagen gel. The gel was pigmented due to melanization. 10th day of culture.

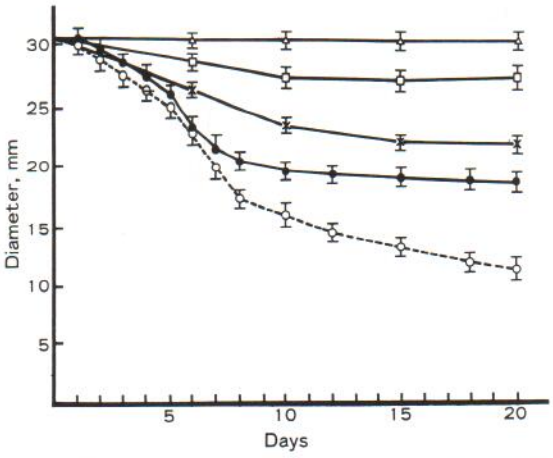

Fig. 3. Time course of collagen gel contraction by B16 melanoma cells without RA (●) and with RA (○), RPMI 1840 Syrian hamster melanoma cell line (×), human melanoma AKI (□) and SEKI (△) line.

tiation in various kinds of cells, gel contraction was enhanced: the area on day 20 accounted for no more than 14% of that on day 0.

Next, several other lines of melanoma were investigated. Gel contraction was not seen in a human SEKI line. When another human-derived AKI line was employed, the contractility of the gel was modest; its area on day 20 was 76.6% of that on day 0. When the RPMI 1846 line from Syrian hamster was used, the area of the gel surface on day 20 was 56.5% of that on day 0. Gel contractility varied among the cell lines used. The viability of each cell line cultured in collagen gel was $89.7 \pm 1.2\%$ (B16, without RA), $90.7 \pm 0.9\%$ (B16, with RA), $90.1 \pm 0.3\%$ (RPMI 1846), $88.6 \pm 1.0\%$ (SEKI) and $88.9 \pm 0.5\%$ (AKI), respectively. The differences between them were not significant.

In all the experiments, the hardness and density of the collagen gels gradually increased, but they never became fragile.

DISCUSSION

Interaction between cancer cells and extracellular matrix has long been an interesting topic, yet much remains unclear about it. Since Sloane et al. (1) reported the correlation between metastatic potential and activities of enzymes relating collagen metabolism, the notice of investigators has been directed to the relationship between the connective tissue and biological and biochemical characteristics of cancer cells (2). Schor et al. (9–12) made a path-finding study of the relation of collagen to the behaviour of melanoma cells; they could apparently migrate and proliferate freely in collagen gel. Bell et al. (4) discovered a peculiar phenomenon, viz., fibroblast-induced contraction of collagen gel. Although the exact mechanism of this contraction is still unknown (5), several explanations have been proposed. The cytoskeleton, including actin filaments, is damaged by cytochalasin B and this reagent inhibited gel contraction completely (4). From this fact it seems incontrovertible that gel contraction is associated with the motility of viable cells. Grinnell & Bennet (13) revealed electronmicroscopically that direct interaction between cells and collagen fibres occurred during culture in collagen gel.

The present investigation has confirmed the contraction of collagen gel (Figs. 2, 3), though neither Schor et al. (9–12) nor Adams & Priestley (14) observed this phenomenon. As shown in Fig. 3, however, this phenomenon occurs much more slowly than fibroblast-induced contraction (4). They probably overlooked this phenomenon, since their experiments were rather short-term ones. Besides, results may vary, depending upon the cell line used.

The question may be raised whether or not collagen gel is eluted by collagenase released by melanoma cells. The collagen gel to which exogenous collagenase was added did not contract and rapidly became fragile. On the other hand, the gel containing melanoma cells contracted to increase in hardness and density. Schor et al. (9, 10) postulated that there might be no relation between collagenase activity and cell movement in the collagen gel and stated that activity of the cells in the gel did not degrade collagen.

It was already shown by us (15) as well as by Adams & Priestley (16) that the fibroblast-induced contraction of collagen gel was promoted by RA. This agent exerted a marked enhancing effect on gel contraction in our experiment using B16 melanoma cells, too (Fig. 3). RA is known as an inducer of differentiation of various cells. In this context, it was reported that RA suppressed the growth of melanoma cells (17). In addition, Buttle & Ehrlich (18) and Steinberg et al. (19) stated that the gel-contracting ability of highly differentiated or normal cells was great. These observations gave a firm basis for the hypothesis that there is a definite relationship between gel contraction and the degree of cell differentiation. It has been reported that RA influence fibronectin synthesis (20). We feel that it is necessary to ascertain whether RA enhances attachment of melanoma cells to collagen or not. But-

tle & Ehrlich (18) postulated the relation of cell morphology in the collagen gel and gel contractility. B16 cells became elongated and some were dendritic in the collagen gel (Fig. 1), but the correlation between cell morphology and contraction is still obscure and this warrants further investigation.

In conclusion, the present observation, showing that collagen gel was contracted by melanoma cells, suggests that interaction takes place between melanoma cells and collagen, and further that a culture system using collagen gel is of value for the study of the interaction between cancer cells and matrix components.

REFERENCES

1. Sloane BF, Honn KV, Sadler JG, Turner WA, Kimpson JJ, Taylor JD. Cathepsin B activity in B16 melanoma cells: A possible marker for metastatic potential. Cancer Res 1982; 42: 980–986.
2. Tryggvason K, Hoeyhtyae M, Salo T. Proteolytic degradation of extracellular matrix in tumor invasion. Biochim Biophys Acta 1987; 907: 191–217.
3. Luikart SD, Maniglia CA, Sartorelli AC. Influence of collagen substrata on glycosaminoglycan production by B16 melanoma cells. Proc Natl Acad Sci USA 1983; 80: 3738–3742.
4. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci USA 1979; 76: 1274–1278.
5. Guidry C, Grinnell F. Contraction of hydrated collagen gels by fibroblasts: Evidence for two mechanisms by which collagen fibrils are stabilized. Collagen Rel Res 1986; 6: 515–529.
6. Oikawa A, Nakayasu M, Claunch C, Tchen TT. Two types of melanogensis in monolayer cultures of melanoma cells. Cell Differ 1972; 1: 149–155.
7. Ishii A, Matsui M, Terano Y, Miura Y. Establishment and characterization of a novel human malignant melanoma cell line AKI. Cell Mol Biol 1988; 34: 255–264.
8. Elsdale T, Bard J. Collagen substrata for studies on cell behavior. J Cell Biol 1972; 54: 626–637.
9. Schor SL. Cell proliferation and migration on collagen substrata in vitro. J Cell Sci 1980; 41: 159–175.
10. Schor SL, Allen TD, Harrison CJ. Cell migration through three-dimensional gels of native collagen fibres: Collagenolytic activity is not required for the migration of two permanent cell lines. J Cell Sci 1980; 46: 171–186.
11. Schor SL, Schor AM, Winn B, Rushton G. The use of three-dimensional collagen gels for the study of tumour cell invasion in vitro: Experimental parameters influencing cell migration into the gel matrix. Int J Cancer 1982; 29: 57–62.
12. Schor SL, Schor AM, Allen TD, Winn B. The interaction of melanoma cells with fibroblasts and endothelial cells in three-dimensional macromolecular matrices: A model for tumor cell invasion. Int J Cancer 1985; 39: 93–102.
13. Grinnell F, Bennet MH. Ultrastructural studies of cell–collagen interactions. Methods Enzymol 1982; 82: 535–544.
14. Adams LW, Priestley GC. Contraction of collagen lattices by skin fibroblasts from dystrophic recessive epidermolysis bullosa and other dermatoses. J Invest Dermatol 1986; 87: 544–546.
15. Kono T, Tanii T, Furukawa M, Kitajima J, Ishii M, Hamada T. Morphological and functional studies of fibroblasts embedded in collagen lattice. Proc Jpn Soc Invest Dermatol 1987; 11: 97–98.
16. Adams LW, Priestley GC. Contraction of collagen lattices by skin fibroblasts: Drug-induced changes. Arch Dermatol Res 1988; 280: 114–118.
17. Lotan R. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochim Biophys Acta 1980; 605: 33–91.
18. Buttle DJ, Ehrlich HP. Comparative studies of collagen lattice contraction utilizing a normal and a transformed cell line. J Cell Physiol 1983; 116: 159–166.
19. Steinberg BM, Smith K, Colozzo M, Pollack R. Establishment and transformation diminish the ability of fibroblasts to contract a native collagen gel. J Cell Biol 1980; 87: 304–308.
20. Bolmer SD, Wolf G. Retinoids and phorbol esters alter release of fibronectin from enucleated cells. Proc Natl Acad Sci USA 1982; 79: 6541–6545.