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Effect of Retinyl Acetate on the Assembly of the Fibronectin Extracellular Matrix and the Processing of the Fibronectin Receptor β Subunit of Confluent C3H/10T1/2 Mouse Embryo Fibroblasts

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Abstract The mouse embryo fibroblast cell line, C3H/10T1/2, synthesized and deposited a large amount of fibronectin especially in the pericellular matrix. Confluent cultures of these cells cultured in the presence of 0.3 μg/ml of retinyl acetate released cell surface fibronectin and the extracellular matrix fibronectin fibrils were disorganized. The immunoblot analysis demonstrated that the number of the fibronectin receptor was decreased in the prolonged culturing of retinyl acetate-treated cells. Immunoprecipitation of ^{35}S-methionine pulse-chase labeled cell extracts by antifibronectin receptor antibody indicated that about one-half of the pre-β subunit was processed and converted to the mature form in control cells, and only about one-fourth of the pre-β subunit was processed in the retinyl acetate-treated confluent cells. 1-deoxymannojirimycin (MNJ), which is an inhibitor of oligosaccharide processing, induced disorganization of the extracellular matrix fibronectin assembly similar to that observed with retinyl acetate. The results of this study suggest that a mechanism of action of retinyl acetate is inhibition of the glycosylation during processing of the fibronectin receptor, a step necessary for fibronectin binding and for assembly of the extracellular matrix.

Key words: retinyl acetate, fibronectin extracellular matrix, fibronectin receptor, β subunit

The interaction of cells with the extracellular matrix molecules is important in many biological processes. For example, the process of malignant transformation involves altered interactions of extracellular matrix molecules with cell surface receptors [Liotta et al., 1986]. One of the main extracellular matrix proteins is fibronectin. Fibroblasts interact with extracellular fibronectin through a specific receptor, which is a member of the integrin family [Juliano, 1987]. This receptor, consisting of α (145-kDa) and β (125-kDa) subunits in mammals, recognizes the Arg-Gly-Asp sequence in fibronectin [Pierschbacher and Ruoslahti, 1984; Yamada, 1989]. The expression and distribution of the fibronectin receptor are strongly related to cell function. Inhibiting the fibronectin receptor function with synthetic peptides containing the Arg-Gly-Asp sequence suppresses fibronectin binding and migration of embryonal, epithelial [Savagner et al., 1986], and tumor cells [Humphries et al., 1986; Gehlsen et al., 1988]. Transforming growth factor β-1 stimulates fibronectin receptor synthesis in human fetal lung fibroblast cells (IMR-90) [Roberts et al., 1988]. The phorbol ester tumor promotor, 12-O-tetradecanoylphorbol 13-acetate (TPA) induces receptor expression but reduces cell adhesion to fibronectin in the K562 erythroleukemia cell line [Symington et al., 1989].

Retinoids are also known to modulate cell morphology. Retinyl acetate causes nearly 90% of C3H/10T1/2 cells to have a very flat morphology, while TPA causes cell rounding [Bertram et al., 1981; Mordan et al., 1984]. In this paper, we report the effect of retinyl acetate on extracellular matrix formation, especially fibronectin assembly. Since exposure of C3H/10T1/2 cells to retinyl acetate after confluence resulted in the separation of fibronectin from the cell surface, we also determined changes in the fibronectin

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receptor synthesis in control and retinyl acetate-treated cells.

MATERIALS AND METHODS

Chemicals

Retinyl acetate (RAC), basal medium Eagle's (BME), methionine free Dulbecco's modified Eagle's medium (DMEM), and 1-deoxymannojirimycinin (MNJ) were from Sigma, and fetal bovine serum (FBS) was obtained from HyClone. Rabbit polyclonal antifibronectin and antifibronectin receptor antibody were obtained from Telios and fluorescein-conjugated and horseradish peroxidase (HRP)-conjugated rabbit IgG were obtained from Calbiochem.

Cell Culture

The C3H/10T1/2 cell line (a gift from J.L. Mordan) was maintained in BME supplemented with 10% FBS in the presence or the absence of 0.3 μg/ml of retinyl acetate in acetone.

Immunofluorescence

Cells were seeded on glass coverslips (Bellco) and cultured. Prior to treatment with antibody, the cells were fixed with methanol at room temperature for 10 min. The fixed cells were incubated with antifibronectin antibody (1:200 dilution in phosphate-buffered saline (PBS)) for 1 h at 37°C in a humidified chamber. After rinsing with PBS, the cells were incubated with fluorescent-labeled secondary antibody for 1 h. Cells were photographed using a Zeiss Photomicroscope (ICM405) and Kodak Tmax 400 film.

Immunoblotting

Cells were solubilized on ice with extraction buffer (10 mM Tris, pH7.6, 0.1 mM NaCl, 20 mM EDTA, 0.5% (w/v) CHAPS), and extracts were centrifuged at 15,000*g* for 15 min. The supernatants were separated by SDS–gel electrophoresis [Laemmli, 1970] and electrophoretically transferred to nitrocellulose membranes. The nitrocellulose membranes were incubated in a blocking solution (5% skim milk in PBS, pH7.4) at 4°C for 12 h. The membranes were then incubated with antifibronectin receptor antibody (1:200 dilution) in the same buffer overnight at 4°C. The nitrocellulose membranes were washed three times with the same blocking solution and incubated with HRP-conjugated secondary antibody (1:20,000 dilution) in the same buffer for 30 min at room temperature. The membranes were washed three times and analyzed with an ECL Western blotting detection system (Amersham).

Pulse-Labeling and Immunoprecipitation

Cells were preincubated with methionine-free DMEM for 30 min and labeled with 200 μCi/ml trans-^{35}S-Label (ICN) in 90% methionine-free DMEM plus regular BME for 2 hs. The labeling medium was replaced with regular BME and incubated for varying lengths of time. Immunoprecipitations were performed essentially as described by Symington et al. [1989]. Aliquots of extracts from cells grown in ^{35}S-methionine were precipitated with 10% trichloroacetic acid and normalized to determine total incorporated radioactivity. The remainder of the supernatant was preadsorbed with protein-A Sepharose CL-4B and Sepharose CL-4B. The nonspecific binding was removed by centrifugation, and the supernatant was incubated with antifibronectin receptor antibody at 4°C. The antigen–antibody complex was precipitated by adding protein–A Sepharose CL-4B. The complex was rinsed three times with extraction buffer and once with washing buffer 1 (10 mM Tris, pH 7.5, 1 M $MgCl_2$) and once with washing buffer 2 (20 mM Tris, pH 7.5, 0.5% (w/v) Nonidet P-40). Samples were eluted with SDS sample buffer and electrophoresed on SDS 7.5% polyacrylamide gels. Gels were fixed and incubated with Amplify (Amersham) following the manufacturer's instructions. Dried gels were quantitated using an AMBIS Radioanalytic Imaging System and autoradiographed using Kodak X-Omat AR film at −70°C.

Glycosylation Analysis

This analysis was performed with the Glycan Differentiation Kit (Boehringer Mannheim Biochemica). In this procedure, immunoprecipitated fibronectin receptor electrophoresed by SDS–polyacrylamide gel was transferred electrophoretically to nitrocellulose membranes (Bio-Rad). The membranes were incubated with the digoxigenin-labeled lectins. Immunoreactions were detected by using alkaline phosphatase-coupled antidigoxigenin.

RESULTS

Figure 1 shows fibronectin immunofluorescence of C3H/10T1/2 cells. C3H/10T1/2 cells synthesize an abundant fibronectin-rich pericellular matrix. The staining pattern of retinyl

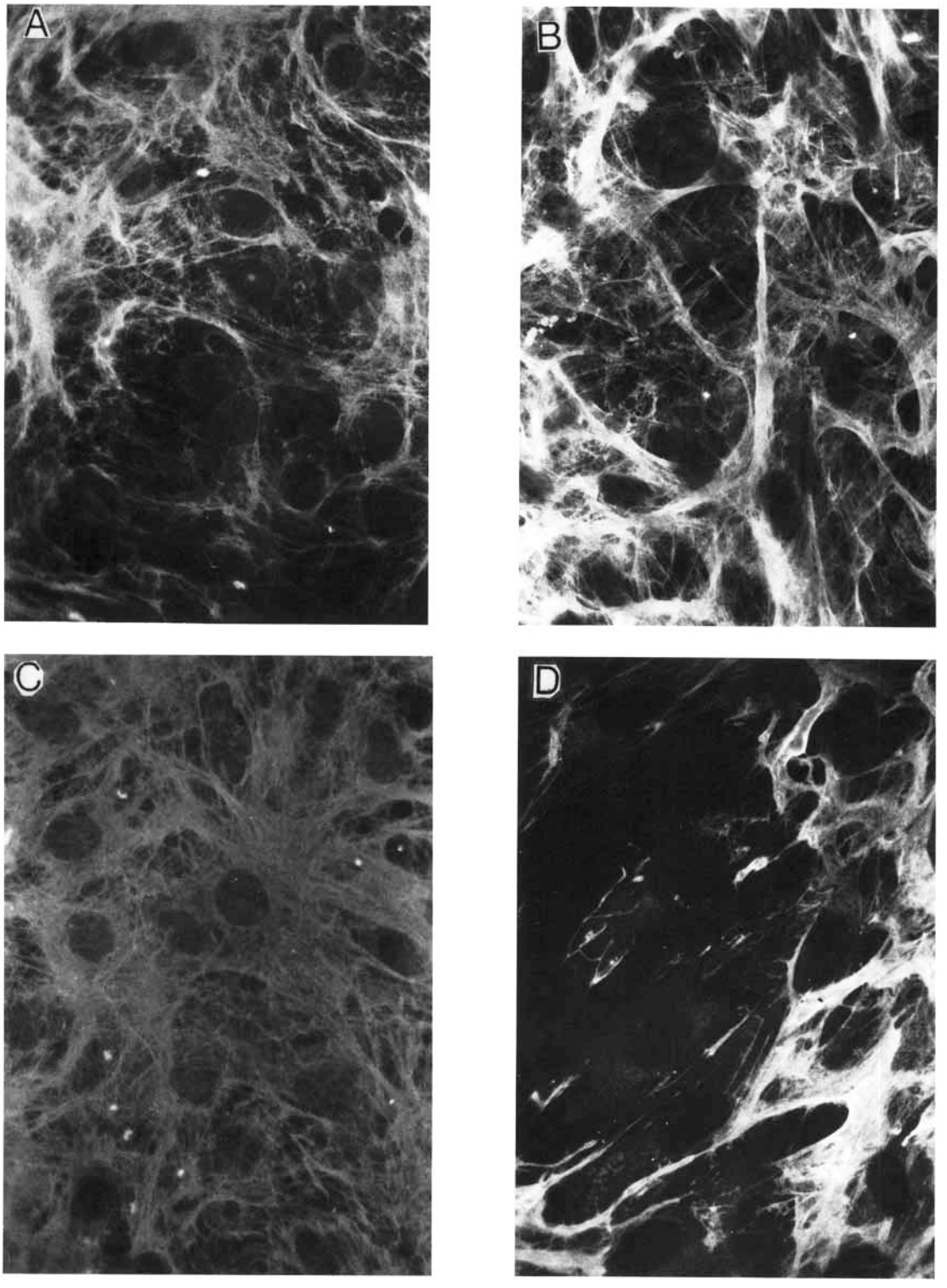

Fig. 1. Distribution of fibronectin in C3H/10T1/2 cells. C3H/10T1/2 cells, cultured in the absence (**A,C**) and presence (**B,D**) of 0.3 μg/ml of retinyl acetate, were fixed just before (**A,B**) and after the confluent stage (**C,D**). Prolonged culture (1 week after the confluent) of C3H/10T1/2 cells in the presence of retinyl acetate resulted in fibronectin matrix assembly disorganization. ×400.

acetate treated C3H/10T1/2 cells was indistinguishable from that of the control culture before the confluent stage (Fig. 1A,B). Prolonged culture of C3H/10T1/2 cells in the presence of retinyl acetate, however, caused loss of the fine cell surface fibronectin fibrils (Fig. 1D), although a widespread fibronectin network structure was still apparent in control cultures (Fig. 1C). The same immunofluorescence staining was observed whether the cells were methanol-fixed (as in Fig. 1), or whether glutaraldehyde (0.01%) or formaldehyde (2%) fixation was used (not shown).

Fibronectin binding to the cell surface is mediated through the fibronectin receptor. Therefore, we traced the amount of fibronectin receptor before and after the confluent stage. Immunoblot analysis showed that the antifibronectin receptor antibody recognized at least two major components under nonreduced conditions (Fig. 2). The upper band is about 125 kDa and the lower band is about 105 kDa, identified as β and pre-β subunits [Akiyama and Yamada, 1987]. Before confluence, control cells expressed a large amount of mature β subunit and a small amount of pre-β subunit. Subconfluent retinyl acetate-treated cells expressed a larger amount of precursor form compared to the control. After confluence, control cells did not significantly alter the ratio between the mature and precursor forms compared with that of the preconfluent stage. In contrast, confluent retinyl acetate treated cells expressed a greatly reduced amount of fibronectin receptors (both mature and precursor) compared to the control, and fibronectin network structure was observed to be disorganized at this stage (Fig. 1D).

The rates of biosynthesis and processing of fibronectin receptors of C3H/10T1/2 cells in the presence or absence of retinyl acetate were measured by using a pulse-chase labeling technique. Confluent cells were labeled with ^{35}S-methionine for 2 h and incubated for varying times in regular growth medium. The kinetic data for the biosynthesis of the fibronectin receptor showed a major difference between control and retinyl acetate-treated cells in the processing of the β subunit from the precursor to the mature form (Fig. 3). The precursor forms of the β subunit in both control and retinyl acetate-treated cells were synthesized at about the same rate. Processing started 2 h after the chase and the precursor forms were present 12 h after ^{35}S-methionine was removed in both control and retinyl acetate-treated cells. The processing pattern was, however, different. About one-half of the pre-β form was processed in control cells (Fig. 3, lower A), while only about one-fourth of the pre-β form was processed in retinyl acetate-treated cells (Fig. 3, lower B).

One possible explanation of these results is that the processing of the β subunit is somehow blocked by the retinyl acetate treatment. In order to investigate this possibility, we treated C3H/10T1/2 cells with an inhibitor of the maturation of the β subunit of the fibronectin receptor and looked at the morphology of the cells. 1-Deoxymannojirimycin (MNJ), which is an inhibitor of Golgi α-mannosidase I, has been reported to block the maturation of the fibronec-

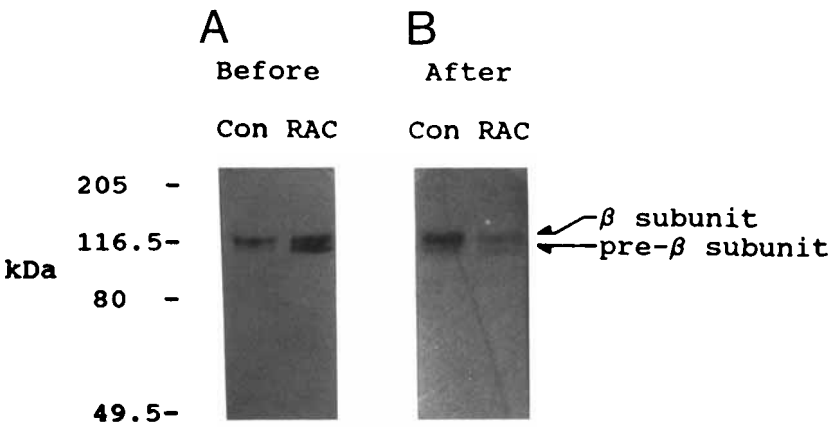

Fig. 2. Immunoblotting of C3H/10T1/2 cells with antifibronectin antibody. C3H/10T1/2 cells were extracted with CHAPS buffer just before (A) and after (B) confluent stage. Cell extracts were separated by 7.5% SDS–PAGE under nonreducing conditions, transferred to nitrocellulose, and blotted with antifibronectin receptor antibody as described under Materials and Methods. The positions of molecular-size standards are also indicated on the left.

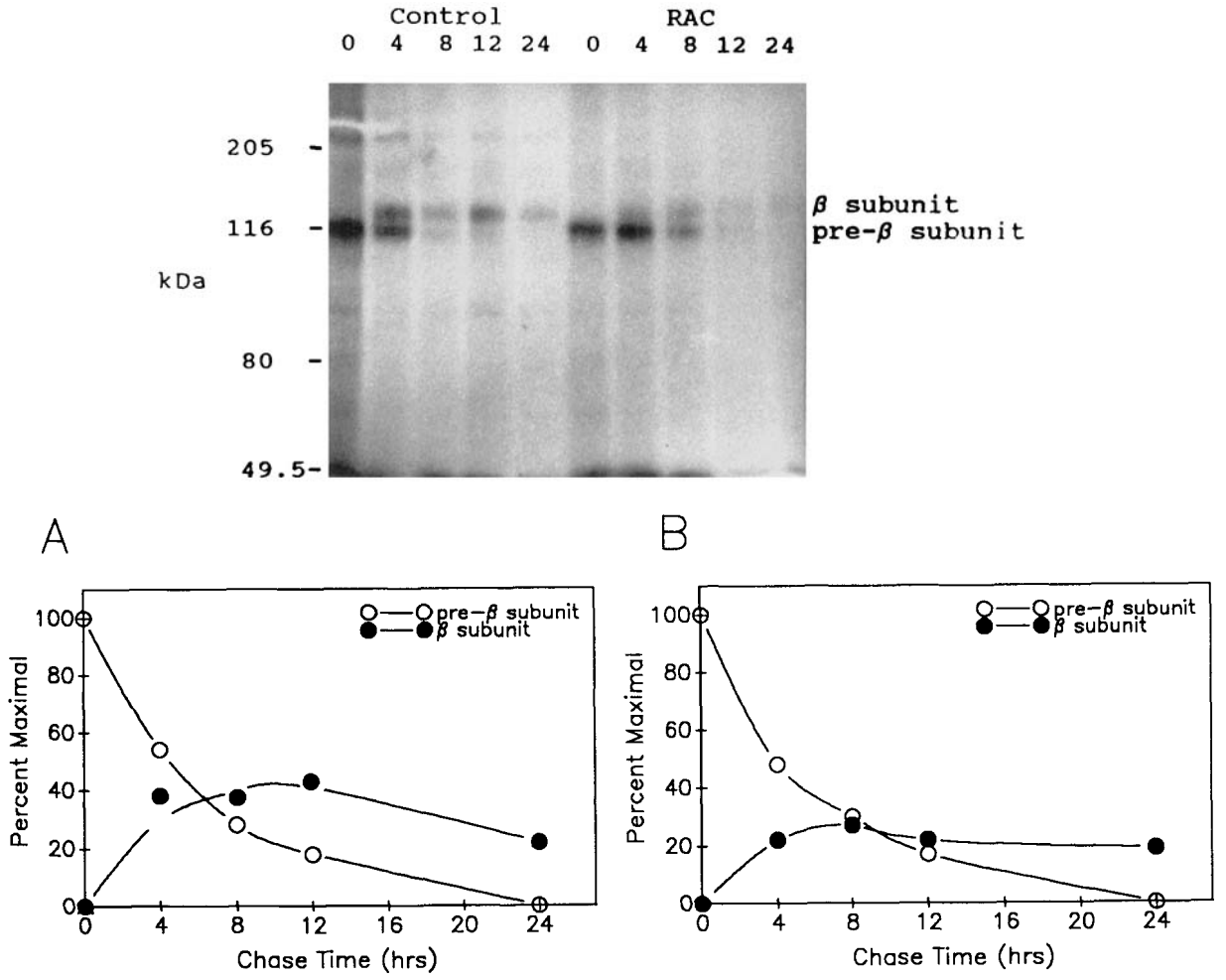

Fig. 3. Effect of retinyl acetate on the processing of the fibronectin receptor complex Upper, autoradiogram of immunoprecipitations of ^{35}S-methionine pulse-chase labeled C3H/10T1/2 cell extracts Mouse embryo fibroblast C3H/10T1/2 cells were pulse labeled with 200 μCi/ml trans ^{35}S for 2 h Cell extracts were immunoprecipitated with antifibronectin receptor antibody and separated by 7 5% SDS–PAGE under nonreducing conditions The positions of molecular size standards are also indicated on the left Lower, quantitation of autoradiogram was determined by AMBIS and the relative radioactivities of the pre-β and β subunits were plotted as a function of the chase time **A:** control **B:** Retinyl acetate-treated cells

tin receptor [Akiyama et al., 1989]. In that study, it was observed that human foreskin fibroblast cells cultured with 200 μg/ml MNJ synthesized only the immature precursor form of both the α and β subunits of the fibronectin receptor. When C3H/10T1/2 cells were treated with 200 μg/ml MNJ for 2 days, cell surface fibronectin became depleted and the extracellular matrix became disorganized (Fig. 4E), whereas non-MNJ-treated cells had a widespread fibronectin network structure (Fig. 4A). These changes were accompanied by inhibition of the maturation of the β subunit of the fibronectin receptor, as shown in Figure 5. This inhibition was concentration dependent (12.5–200 μg/ml MNJ). When the cells were treated with 100 or 200 μg/ml MNJ, the processing from pre-β to β form of fibronectin receptor was completely inhibited (lanes 5 and 6) and 25–50 μg/ml MNJ partially inhibited the processing (lanes 3 and 4).

Since both retinyl acetate and MNJ inhibited the maturation of the fibronectin receptor, we assumed that the role of retinyl acetate is to modify glycosylation in the maturation process of the fibronectin receptor. Therefore, we examined possible changes in the fibronectin receptor induced by retinyl acetate treatment. Our preliminary result, however, did not show any significant differences in sialic acid, mannose, and acetylglucosamine contents in the fibronectin receptor between the control and retinyl acetate-treated cells (data not shown).

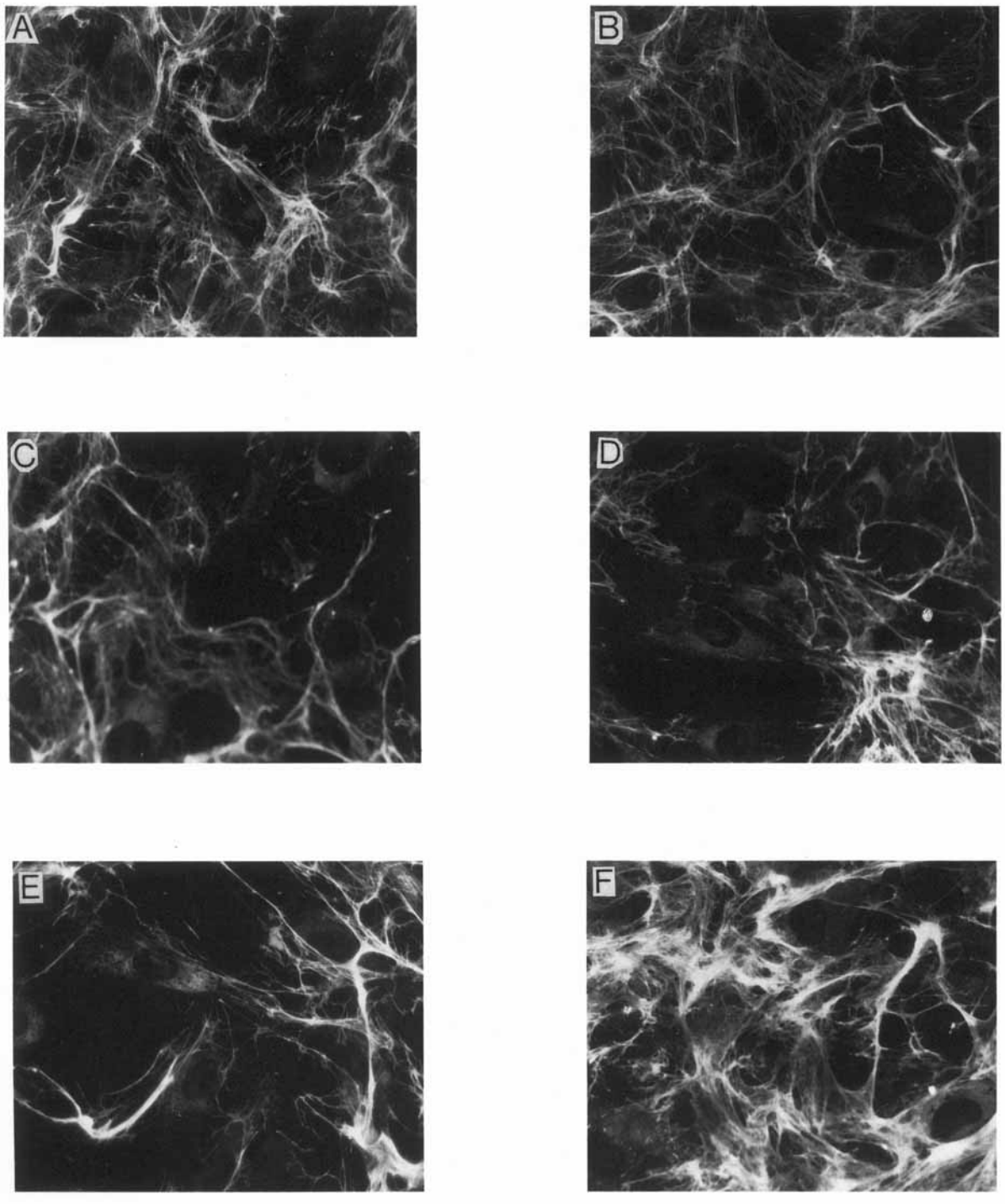

Fig 4 Effect of MNJ on fibronectin matrix assembly C3H/10T1/2 cells cultured in the regular medium for 2 days were treated with 0 μg/ml (**A**), 12 5 μg/ml (**B**), 50 μg/ml (**C**), 100 μg/ml (**D**), 200 μg/ml of MNJ (**E**), and 0 3 μg/ml (**F**) of retinyl acetate for 2 days The cells were then fixed and stained as described under Materials and Methods Treating C3H/10T1/2 cells with MNJ reduced the fibronectin matrix assembly and was MNJ concentration dependent ×400

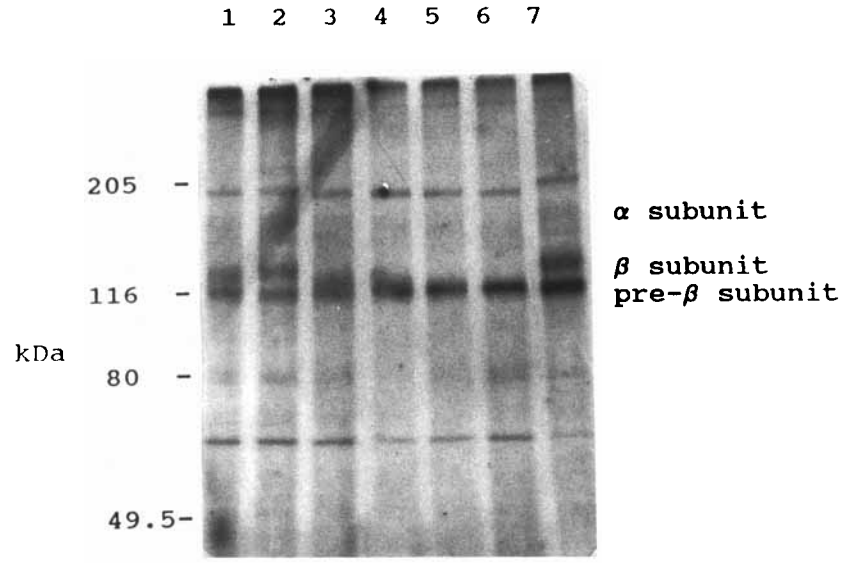

Fig. 5. Comparison of fibronectin receptors from control cells, MNJ-treated cells, and retinyl acetate-treated cells Mouse embryo fibroblast C3H/10T1/2 cells were first cultured in the absence (**lane 1**) or presence of 12 5 μg/ml (**lane 2**), 25 μg/ml (**lane 3**), 50 μg/ml (**lane 4**), 100 μg/ml (**lane 5**), 200 μg/ml of MNJ (**lane 6**), for 1 day Then, 100 μCi/ml 35S-methionine was added and cells were incubated overnight, **lane 7:** cells cultured with 0 3 μg/ml of retinyl acetate Cell extracts were immunoprecipitated with antifibronectin receptor polyclonal antibody and electrophoresed in 7 5% SDS-PAGE under nonreducing condition The positions of molecular-size standards are also indicated on the left

DISCUSSION

Fibroblasts interact with extracellular fibronectin through a specific receptor, which has been reported to be important in extracellular matrix assembly [McDonald et al., 1987; Roman et al., 1989]. The expression and distribution of the fibronectin receptor influence cell function. We have observed fibronectin release from the cell surface and the disorganization of the extracellular matrix fibronectin network structure when mouse embryo fibroblast, C3H/10T1/2 cells, were cultured in the presence of retinyl acetate. This phenomenon occurred after confluence. The immunoblot results showed that the amount of the immature form of fibronectin receptor β was increased by the retinyl acetate treatment. We believe that any one of the following possible mechanisms might explain these results: (1) retinyl acetate activates biosynthesis of the fibronectin receptor, (2) retinyl acetate slows down turnover of the fibronectin receptor, and (3) retinyl acetate modifies the processing of the fibronectin receptor. Pulse-chase labeling experiments indicated that biosynthesis of the fibronectin receptor β subunit was not influenced by retinyl acetate treatment. However, about one-half of the pre-β subunit was converted to mature form in the nontreated control cells, in contrast to, one-quarter of the pre-β subunit being converted to mature form in retinyl acetate-treated cells. Thus, it would seem that the third mechanism listed above, namely an alteration in receptor processing, is likely.

One possible effect of retinyl acetate on receptor processing is to alter glycosylation. Many membrane proteins are glycoproteins that carry asparagine-linked (N-linked) oligosaccharides. Biosynthesis of N-linked glycoproteins is processed through a high-mannose intermediate. The mannose residues are removed by mannosidases and more complex oligosaccharides are transferred during the processing [Hubbard and Ivatt, 1981]. The pre-β subunit of the fibronectin receptor contains asparagine-linked high mannose oligosaccharides [Akiyama and Yamada, 1987]. MNJ, which is a mannose analog, blocks the removal of mannose residues from asparagine-linked oligosaccharides [Bishoff and Kornfed, 1984; Fuhrmann et al., 1984, 1985; Hughes et al., 1987] and has been reported to be an effective inhibitor of the maturation of the fibronectin receptor in human fibroblasts [Akiyama et al., 1989]. As shown in Figure 4, treatment of C3H/10T1/2 cells with MNJ inhibited fibronectin receptor β subunit processing. This inhibition may cause fibronectin release from the cell surface and conversion of a fine fibronectin network to a disorganized pattern of cable-

like structure, which was very similar to those observed in retinyl acetate treated-cells. The maturation of the fibronectin receptor is reported to be required for fibronectin binding activity [Akiyama et al., 1989]. Our data are consistent with this finding.

Retinoids have been reported to increase the activity of galactosyltransferase [Creek and Morré 1980] and sialyltransferase [Deutsch and Lotan 1983; Lotan et al., 1983]. Despite possible differences in oligosaccharide composition of the fibronectin receptor of retinyl acetate-treated cells, our preliminary data indicate that retinyl acetate has no effect on the contents of sialic acid, mannose, and acetylglucosamine in the fibronectin receptor. Since the sensitivity of proteins to proteases depends on the degree of their glycosylation [Loh and Gainer 1978], it would be possible that in the retinyl acetate-treated group the amount of mature fibronectin receptor is decreased through proteolysis of intermediates without an appreciable change in the composition of polysaccharides of the mature receptor.

The reason that retinyl acetate effects on fibronectin receptors occur only after the confluent stage is unknown. The nuclear receptors for retinoic acid have been cloned [Petkovich et al., 1987; Giguere et al., 1987; Brand et al., 1988; Benbrook et al., 1988; Zelent, 1989]. The retinoic acid receptors are ligand-dependent transcriptional factors and regulate gene expression by binding to a short DNA sequence, called the retinoic acid-responsive element. Recently, Schüle et al. [1990] showed that the retinoic acid receptor has an antagonistic effect to the Jun–Fos on the human osteocalcin gene. Thus, in the glycosylation processing of the fibronectin receptor, one of the glycosyltransferase genes might possess one of these transcriptional factor binding sites. After confluence the transcriptional factor, which is antagonistic to the retinoids, might be reduced thereby allowing the effect of retinyl acetate to become dominant.

Besides the modification of fibronectin receptor glycosylation, Meromsky and Lotan [1984] reported that labeled glucosamine incorporation into secreted fibronectin was decreased by retinyl acetate treatment in cultured human chondrosarcoma and osteosarcoma cells. Bernard et al. [1984] reported that fibronectin secreted into the culture medium of retinoic acid treated chondrocytes consists of more complex type oligosaccharides than fibronectin released from untreated cells. Therefore, fibronectin itself might be modified by the retinyl acetate treatment, which might also influence the assembly of the extracellular matrix fibronectin fibrils. The increased release of fibronectin from retinyl acetate treated C3H/10T1/2 cells could be due to the result of an increased activity of proteolytic enzymes, such as plasminogen activator, although Lotan et al. [1982] reported that retinoids have no consistent effect on plasminogen activator synthesis of cells.

Transformation of cells is associated with reduced assembly of the extracellular matrix fibronectin [Hynes, 1976; Vaheri et al., 1978]. In order to explain the loss of cell surface fibronectin, Plantefaber et al. [1989] reported that the fibronectin receptor was decreased in several oncogenically transformed cells compared with their normal counterpart. In contrast, Akiyama et al. [1990] reported that normal human fibroblast cells carried a large amount of pre-β subunit which is not present in transformed cells and that there was no major transformation-dependent change in total quantities of the mature fibronectin receptor subunit. These investigators proposed that transformed cells differ in the localization and processing of the fibronectin receptors [Akiyama et al., 1990]. Retinoids have been reported to inhibit the growth of some malignant cells [Haussler et al., 1983; Roberts and Sporn, 1984], and it will be interesting to study the processing of the fibronectin receptor in this connection.

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REFERENCES

Akiyama SK, Yamada KM (1987) Biosynthesis and acquisition of biological activity of the fibronectin receptor J Biol Chem 262 17536–17542

Akiyama SK, Yamada SS, Yamada KM (1989) Analysis of the role of glycosylation of the human fibronectin receptor J Biol Chem 264 18011–18018

Akiyama SK, Larjava H, Yamada KM (1990) Differences in the biosynthesis and localization of the fibronectin receptor in normal and transformed cultured human cells Cancer Res 50 1601–1607

Benbrook D, Lernhardt E, Pfahl M (1988) A new retinoic acid receptor identified from a hepatocellular carcinoma Nature 333 669–672

Bernard BA, De Luca LM, Hassell JR, Yamada KM, Olden K (1984) Retinoic acid alters the proportion of high mannose to complex type oligosaccharides on fibronectin secreted by cultured chondrocytes J Biol Chem 259 5310–5315

Bertram JS, Mordan LJ, Blair SJ, Hui S (1981) Effects of retinoids on neoplastic transformation, cell adhesion, and membrane topography of cultured 10T1/2 cells Ann NY Acad Sci 359 218–236

Bischoff J, Kornfeld R (1984) The effect of 1-deoxymannojirimycin on rat liver α-mannosidases Biochem Biophys Res Commun 125 324–331

Brand N, Petkovich M, Krust A, Chambon P, de The H, Marchio A, Tiollais P, Dejean A (1988) Identification of a second human retinoic acid receptor Nature 332 850–853

Creek KE, Morre DJ (1980) Effects of retinol and retinol palmitate on glycolipid and glycoprotein galactosyltransferase activities of rat liver plasma membranes Biochem Biophys Res Commun 95 1775–1780

Deutsch V, Lotan R (1983) Stimulation of sialyltransferase activity of melanoma cells by retinoic acid Exp Cell Res 149 237–245

Fuhrmann U, Bause E, Legler G, Ploegh H (1984) Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides Nature 304 135–139

Fuhrmann U, Bause E, Ploegh H (1985) Inhibitors of oligosaccharide processing Biochim Biophys Acta 825 95–110

Gehlsen KR, Argraves WS, Pierschbacher MD, Ruoslahti E (1988) Inhibition of In Vitro tumor cell invasion by Arg-Gly-Asp-containing synthetic peptides J Cell Biol 106 925–930

Giguere V, Ong ES, Segui P, Evans RM (1987) Identification of a receptor for the morphogen retinoic acid Nature 330 624–629

Haussler MR, Sidell N, Michael K, Donaldson C, Altman A, Mangelsdorf D (1983) Specific high-affinity and biologic action of retinoic acid in human neuroblastoma cell lines Proc Natl Acad Sci USA 80 5525–5529

Hubbard SC, Ivatt RJ (1981) Synthesis and processing of asparagine-linked oligosaccharides Annu Rev Biochem 50 555–583

Hughes RC, Foddy L, Bause E (1987) Asparagine-linked oligosaccharides of BHK cells treated with inhibitors of oligosaccharide processing Biochem J 247 537–545

Humphries M, Olden K, Yamada KM (1986) A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells Science 233 467–470

Hynes RO (1976) Cell surface proteins and malignant transformation Biochim Biophys Acta 458 73–107

Juliano RL (1987) Membrane receptors for extracellular matrix macromolecules, relationship to cell adhesion and tumor metastasis Biochim Biophys Acta 907 261–278

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4 Nature 227 680–685

Liotta LA, Rao CN, Wewer UM (1986) Biochemical interactions of tumor cells with the basement membrane Annu Rev Biochem 55 1037–1057

Loh YP, Gainer H (1978) The role of glycosylation on the biosynthesis, degradation, and secretion of the ACTH-β-lipotropin common precursor and its peptide products Febs Lett 96 269–272

Lotan R, Lotan D, Kadouri A (1982) Comparison of retinoic acid effects on anchorage-dependent growth, anchorage-independent growth and fibrinolytic activity of neoplastic cells Exp Cell Res 141 79–89

Lotan R, Neumann G, Deutsch V (1983) Identification and characterization of specific changes induced by retinoic acid in cell surface glycoconjugates of S91 murine melanoma cells Cancer Res 43 303–309

McDonald JA, Quade BJ, Broekelmann TJ, LaChance R, Forsman K, Hasegawa E, Akiyama SK (1987) Fibronectin's cell-adhesive domain and an amino-terminal matrix assembly domain participate in its assembly into fibroblast pericellular matrix J Biol Chem 262 2957–2967

Meromsky L, Lotan R (1984) Modulation by retinoic acid if cellular, surface-exposed, and secreted glycoconjugates in cultured human sarcoma cells JNCI 72 203–216

Mordan LJ, Hui SW, Bertram JS (1984) Modulation by retinyl acetate of microfilament bundle formation in C3H/10T1/2 cells J Cell Biochem 24 15–25

Petkovich M, Braannd NJ, Krust A, Chambon P (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors Nature 330 444–450

Pierschbacher MD, Ruoslahti E (1984) Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule Nature 309 30–33

Plantefaber LC, Hynes RO (1989) Changes in integrin receptors on oncogenically transformed cells Cell 56 281–290

Roberts CJ, Birkenmeier TM, McQuillan JJ, Akiyama SK, Yamada SS, Chen WT, Yamada KM, McDonald JA (1988) Transforming growth factor β stimulates the expression of fibronectin and of both subunits of the human fibronectin receptor by cultured human lung fibroblast J Biol Chem 263 4586–4592

Roberts AB, Sporn MB (1984) Cellular biology and biochemistry of the retinoids In Sporn MB, Robert AB, Goodman DS (eds) "The Retinoids" Orlando, Florida Academic Press, Vol 2, pp 209–286

Roman J, LaChance RM, Broekelmann TJ, Kenedy CJR, Wayner EA, Carter WG, McDonald JA (1989) The fibronectin receptor is organized by extracellular matrix fibronectin, implications for oncogenic transformation and for cell recognition of fibronectin matrices J Cell Biol 108 2529–2543

Savagner P, Imhof BA, Yamada KM, Thiery JP (1986) Homing of hemopoietic precursor cells to the embryonic thymus Characterization of an invasive mechanism induced by chemotactic peptides J Cell Biol 103 2715–2727

Schule R, Umesono K, Mangelsdorf DJ, Bolado J, Pike JW, Evans RM (1990) Jun–Fos and receptors for Vitamin A and D recognize a common response element in the human osteocalcin gene Cell 61 497–504

Symington BE, Symington FW, Rorshneider LR (1989) Phorbol ester induces increased expression, altered glycosylation, and reduced adhesion of K562 erythroleukemia cell fibronectin receptor J Biol Chem 264 13258–13266

Vaheri A, Mosher DF (1978) High molecular weight, cell surface-associated glycoprotein (fibronectin) lost in malignant transformation Biochim Biophys Acta 516 1–25

Yamada KM (1989) Fibronectin domains and receptors In Mosher DF (ed) "Fibronectin" Orlando, Florida Academic Press, pp 47–121

Zelent A, Krust A, Petkovich M, Kastner P, Chambon P (1989) Cloning of murine α and β retinoic acid receptors and a novel receptor γ predominantly expressed in skin Nature 339 714–717