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Biosynthesis and Secretion of Fibronectin in Human Melanoma Cells

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The biosynthesis and secretion of cellular fibronectin from human melanoma cells have been investigated by pulse-chase/immunoprecipitation analysis. Melanoma cells synthesize endoglycosidase H (Endo H)-sensitive glycoprotein precursors of fibronectin glycoproteins which chase to an Endo H-resistant monomer with an apparent M_r of 240,000 (240 K). This molecule, which has a significantly higher molecular weight than normal plasma or cellular fibronectin, is rapidly secreted by melanoma cells, resulting in the secretion of 80% of newly synthesized fibronectin in 120 min, following a 10-min biosynthetic pulse. This active secretory process can be inhibited by brief exposure of melanoma cells to sodium monensin (10^{-7} M), which also results in a modified fibronectin of lower apparent M_r. Monosaccharide-incorporation studies of melanoma fibronectin reveal that monensin significantly inhibits galactose and fucose incorporation into this glycoprotein, correlating with reported effects of monensin on Golgi apparatus functions. These studies indicate that this tumor-associated and biosynthetically altered cellular fibronectin is a rapidly secreted major N-linked glycoprotein of metastatic human melanoma cells.

Key words: biosynthesis, secretion, melanoma, fibronectin

Fibronectin is a major high molecular weight glycoprotein found at the cell surfaces of normal cells, in the circulation, and in a variety of tissues [for review see 1–4]. Significant progress has been made in defining the structure of the fibronectin molecule in relationship to functional domains such as binding sites for collagen, heparin, fibrinogen, and molecules associated with cell attachment functions [5 –11].

The initial observations indicating that transformed and spontaneous tumor cells demonstrated an absence or significant decrease in cell surface fibronectin led to speculations that the presence of this molecule was inversely related to the transformed phenotype [12,13]. Several studies indicated that transformed cells have a reduced rate of fibronectin biosynthesis; however, a number of tumor cell lines

Abbreviations: SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; IAD, immunoadsorbents; PTO, phosphate-buffered saline pH 7.2/0.5% Tween 20/0.1% ovalbumin; Endo H, endoglycosidase H.

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originating from different species seem to synthesize fibronectin, but either retain these molecules intracellularly or secrete them into the extracellular media without incorporating them into the extracellular matrix [14,15]. Since the precise molecular events of fibronectin biosynthesis have not been investigated extensively in spontaneous human tumor cell systems, we chose to study this process in human melanoma cell lines derived from secondary metastatic lesions because of their highly metastatic nature and apparent cell-surface expression of fibronectin [16]. We report here on the biosynthesis, cellular expression, and secretion of fibronectin in human melanoma cells.

MATERIALS AND METHODS

Cell Lines

The M21 and M14 CDM lines were derived from metastatic lesions of melanoma patients and adapted to in vitro tissue culture by Morton and colleagues at UCLA [17]. The M21 cell line was maintained in RPMI 1640 medium supplemented with 10% calf serum (GIBCO, Grand Island, New York), 50 μg/ml gentamycin sulfate, and 4 mM glutamine in a 5% CO_2 atmosphere. The M14 CDM cell line, originally adapted to grow in serum-free media devised by Chee and colleagues [18], was adapted by us to grow in a commercially available serum free synthetic medium, Synmed (Centaurus, Inc).

Biosynthetic Labeling and Pulse-Chase Experiments

All long-term intrinsic labeling experiments of M21 melanoma cells with glycoprotein precursors were performed as previously described [24]. In labeling experiments with monosaccharides, M21 cells were intrinsically radiolabeled in glucose-free Selectamine RPMI 1640 media (GIBCO), supplemented with 10% dialyzed fetal calf serum. Pulse-chase studies were performed by first starving M21 melanoma cells (2×10^6 cells/ml) for 30 min in methionine-free RPMI 1640 Selectamine media (GIBCO) supplemented with 10% dialyzed fetal calf serum. ^{35}S-methionine (1 mCi) was then added to initiate a 10-min pulse. After a 1-min centrifugation step at 1,000g, the pulsed cells were resuspended in nonradioactive chase media containing an 8 log excess of cold methionine over the original pulse of ^{35}S-methionine. Aliquots (1 ml) were removed at the times indicated, detergent extracts of cells or spent media were subjected to indirect immunoprecipitation, and subsequent analysis of the precipitates was performed on SDS-PAGE. In some experiments, M21 cells were preexposed to 10^{-7} monensin (Calbiochem, LaJolla, California), a monovalent, cationic ionophore that can inhibit cell surface expression and intracellular transport of glycoproteins [19–22]. Exposure to monensin was for 2 h prior to pulse-chase intrinsic labeling, or for 18 h prior to long-term intrinsic labeling. Protein synthesis was not affected under these conditions of monensin exposure.

Indirect Immunoprecipitation Analysis and SDS-Polyacrylamide Gel Electrophoresis SDS-PAGE

A rabbit antiserum, 1450 R5, which was produced with isolated human plasma fibronectin [23], was used to form Sepharose protein-A (Sigma) immunoadsorbents (IAD) for the indirect immunoprecipitation of biosynthetically labeled fibronectin.

Serological analysis with 1450 R5 revealed that it will specifically bind and immunoprecipitate plasma fibronectin, and that this antiserum does not react with cell surfaces of fibronectin-negative cell types such as human erythrocytes and Epstein-Barr virus–transformed lymphoblastoid cell lines autologous to the melanoma cells under study. In addition, antiserum 1450 R5 demonstrates no serological activity with melanoma spent media that were depleted of fibronectin by gelatin-Sepharose affinity chromatography, indicating high specificity for melanoma cellular fibronectin. Aliquots of 1450 R5 were initially preadsorbed to a 10% (vol/vol) Sepharose protein-A suspension in PTO (phosphate-buffered saline, pH 7.2/0.5% Tween 20/0.1% ovalbumin) for 2 hr at 4°C with rotation. The IAD was then washed three additional times with PTO by centrifugation at 1,000g, followed by an overnight incubation with either biosynthetically labeled spent medium or RIPA (0.01 M Tris HCl/0.15 M NaCl/1% Triton X-100/1% deoxylcholate/0.1% sodium dodecyl sulfate/1% Trasylol) detergent extracts of M21 melanoma cells. These immunoadsorbents were then washed three additional times prior to elution and subjected to SDS-PAGE analysis with molecular weight standards as previously described [24]. In some pulse-chase experiments washed immunoprecipitates of fibronectin were digested prior to analysis by SDS-PAGE for 1 hr at 37°C in 20 mM sodium citrate buffer, pH 5.5, containing 0.01 units of endoglycosidase H (endo-β-N-acetylglucosaminidase H purified from Streptomyces plicatus by the method of Tarentino et al [25] and obtained from Health Research, Albany, New York).

Fluorography of SDS-PAGE slab gels was performed according to Bonner and Laskey [26], and in some experiments fluorograph patterns were quantitatively analyzed by a Zeineh soft laser densitometer.

Serological Assays

Fibronectin was analyzed on melanoma cell surfaces and in solid phase utilizing a radioimmunometric binding assay developed in this laboratory with ^{125}I-protein A [27].

Materials

L-^{35}D-methionine (1,035 Ci/mmol), L-4,5-^{3}H(N)-leucine (42 Ci/mmol), D-2-^{3}H(N)-mannose (20 Ci/mmol), D-6-^{3}H(N)-glucosamine (30 Ci/mmol), L-6-^{3}H-fucose (40 Ci/mmol), and D-1, ^{3}H(N)-galactose (25 Ci/mmol) were all obtained from New England Nuclear. ^{14}C-labeled molecular weight standards used for SDS-PAGE were ^{14}C-myosin, ^{14}C-phosphorylase B, ^{14}C-albumin, and ^{14}C-ovalbumin, which were also obtained from New England Nuclear. All other chemicals were reagent grade or better. Protein determinations were made according to the method of Lowry et al [28]. We gratefully acknowledge the purified plasma fibronectin obtained from Dr Mark Ginsberg, Research Institute of Scripps Clinic. In some experiments, melanoma fibronectin was isolated from the spent tissue culture media of the M14 CDM cell line by affinity chromatography on gelatin-Sepharose [4].

RESULTS

The initial experiments were designed to examine several melanoma cell lines for cell-surface expression of fibronectin as previous results from this laboratory

indicated that human melanoma cells secreted fibronectin [23]. Table I summarizes the results of a radioimmunometric binding assay for cell-surface and secreted fibronectin with two human melanoma cell lines: M21 and M14 CDM. Both cell lines demonstrated specific antigenic activity of cell-surface fibronectin. In the case of the M14 CDM cell line, this cell-surface antigenic activity cannot be caused simply by absorption of cross-reactive bovine fibronectin to the cell surface, because this cell line has been maintained continuously under serum-free conditions for 4 years. This clearly demonstrates that human melanoma cells do express cell-surface fibronectin. In addition, the M14 CDM cells secrete fibronectin, which can be isolated by gelatin-Sepharose affinity chromatography and is highly reactive in solid-phase radioimmunometric binding assays with the antifibronectin antiserum 1450 R4 (Table I).

We next examined the molecular specificity of antiserum 1450 R5 by coupling indirect immunoprecipitation with SDS-PAGE analysis of intrinsically labeled M14 CDM and M21 cell line spent media components (Fig. 1). Both cell lines secrete a broad component with an apparent M_r of 240 K recognized by antiserum 1450 R5 (Fig. 1). Under these conditions, melanoma fibronectin migrates slower than the plasma fibronectin (220 K) doublet, which was used as a standard in parallel experiments, indicating that tumor cells do secrete a fibronectin molecule of larger molecular weight. This increase in molecular weight cannot simply be attributed to a difference between cellular and plasma fibronectins, since human synovial membrane fibroblast cultures secrete a fibronectin molecule very similar in molecular weight to plasma fibronectin (data not shown).

The next experiments specifically examined the biosynthesis, processing, and secretion of this unusual, high-molecular-weight fibronectin of human melanoma cells. Figure 2 depicts the pulse-chase experiments examining the biosynthesis and processing of N-linked oligosaccharides as judged by sensitivity to Endo H. Detergent lysates of M21 melanoma cell were subjected to indirect immunoprecipitation, Endo H digestion, and SDS-PAGE analysis at the time points indicated (Fig. 2). At the first time points after the pulse (0, 5 min), a prominent component is visible in controls not treated with Endo H with an apparent M_r of 230 K along with several

TABLE I. Radioimmunometric Binding Analysis of Antifibronectin Antiserum 1450 R5

Target	Specific cpm bound[a]
M21 (100,000 cells)	30,680
M14 CDM (100,000 cells)	10,040
M14 CDM FN[b] (2 μg)	115,221

[a]Specific cpm bound equals (cpm bound with antiserum 1450 R5) − (cpm bound with normal rabbit serum) utilizing a radioimmunometric binding assay [27]. The cpm bound by normal rabbit serum did not exceed 2% of the total cpm bound by antiserum 1450 R5 in these samples.

[b]Fibronectin was isolated from concentrated spent medium of M14 CDM lines, grown under serum-free conditions, by affinity chromatography on gelatin-Sepharose [4] and analyzed with a solid-phase radioimmunometric binding assay. Two micrograms (as an approximation on yields from batch cultures) represents a typical 72-hr secretion of fibronectin from 5×10^5 M14 CDM cells.

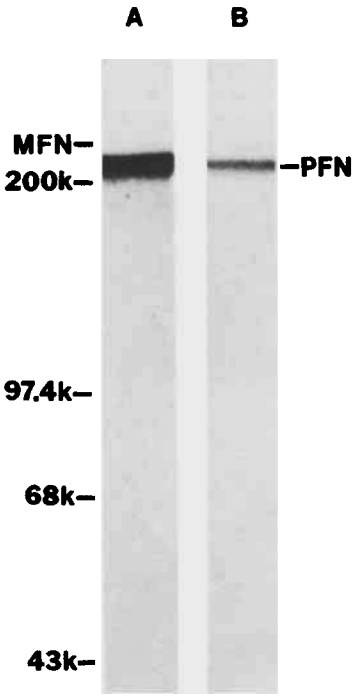

Fig. 1. Immunoprecipitation profile obtained by reacting antiserum 1450 R5 with fibronectin from melanoma cells, intrinsically labeled for 18 hr. Lane A represents the profile of ^{35}S-methionine labeled M21 melanoma cells; lane B shows the profile obtained with ^{3}H-leucine-labeled M14 CDM melanoma cells. The migration of melanoma fibronectin (MFN) and that of the plasma fibronectin (PFN) standard are shown in parallel studies with additional molecular weight markers on this 5% acrylamide SDS-PAGE. The fluorograph represents a 72-hr exposure.

minor, lower-molecular-weight components. A parallel analysis of the Endo H-treated (0+, 5+ min) fibronectin precursors resulted in the visualization of faster-migrating components including a major component with an apparent M_r 220 K. These results indicate that those fibronectin molecules recognized at early time points contain high mannose oligosaccharides that are sensitive to Endo H digestion. At the 25-, 30-, and 45-min control time points, the major bands appear as a doublet which is best visualized at the 45-min time point. The upper band at M_r 240 K appears resistant to Endo H digestion, whereas the lower band migrates faster following Endo H digestion as indicated by treatment with the enzyme at 30-, and 45-min time points. This pattern is consistent throughout the remaining time points, the only obvious difference being the gradual decrease in relative amounts of the two fibronectin components until the 120-min time point is reached. This observation indicates the loss of a large amount of cell-associated fibronectin at early time points in the biosynthesis of fibronectin in melanoma cells.

This study also indicates that there is a rapid synthesis of N-linked glycosylated fibronectin in melanoma cells, culminating in the synthesis of an Endo H-resistant 240 K component and an unusual Endo H-sensitive lower-molecular-weight component at apparent M_r of 235 K which remains sensitive to the enzyme even after 4 h of biosynthetic chase period. The apparent loss of fibronectin components during this experiment, as verified by soft laser densitometric scans of the fluorographs in Figure 2, was a rapid event, and experiments were next performed to examine the kinetics of fibronectin secretion from these human melanoma cells.

Accordingly, the results of parallel pulse-chase studies examining the secretion of soluble fibronectin in the spent medium are shown in Figure 3. Secretion of

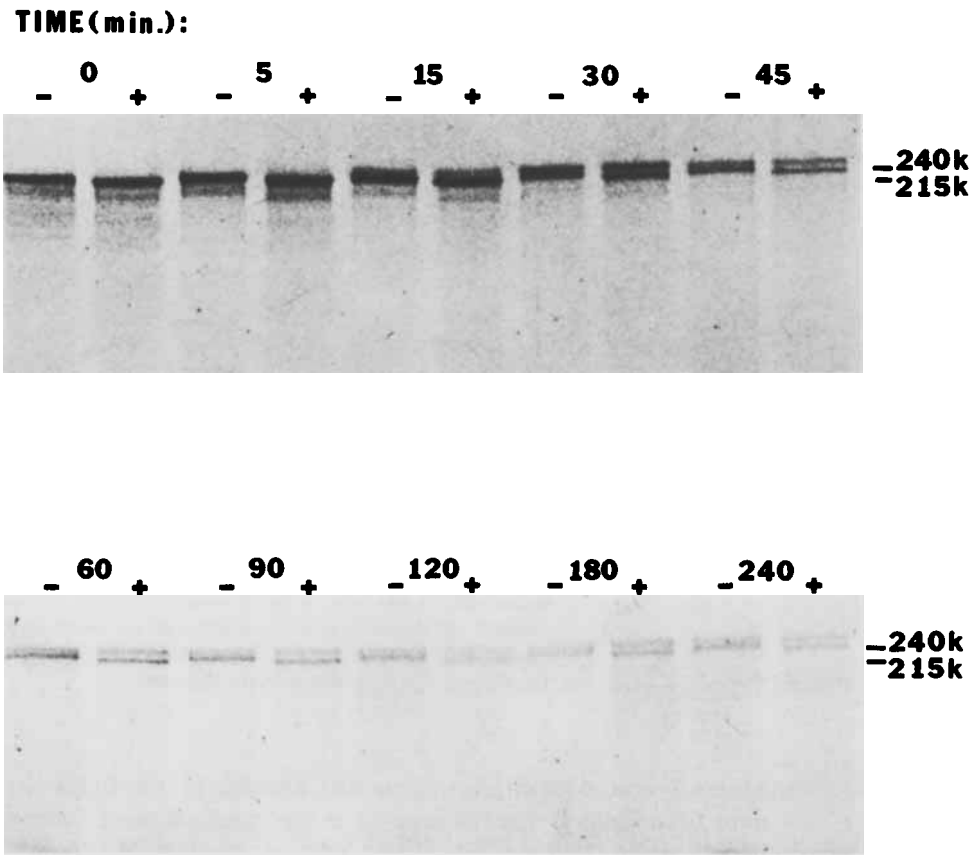

Fig. 2. Pulse-chase biosynthetic analysis of fibronectin in detergent lysates of M21 melanoma cells. M21 melanoma cells, pulsed for 10 min with ^{35}S-methionine, were harvested at the time points indicated in the chase and extracted with detergent lysis buffer. Indirect immunoprecipitates of fibronectin obtained in parallel experiments were either utilized as control (−) or treated with endoglycosidase H (+) prior to analysis on 5% acrylamide SDS-PAGE. The migration of two molecular weight standards from human red blood cell ghost membranes [24] is indicated in this 96-hr exposure fluorograph.

fibronectin is not seen at early time points of 0, 5, and 15 min. However, at 30 min, the first appearance of a 240 K component in the spent medium can be observed in immunoprecipitates (Fig. 3, 30 min). This component increases in intensity until the 120-min time point. These observations indicate that the 240 K component first visualized in detergent lysates from melanoma cells after 15, 30, and 45 min into the chase (Fig. 2) is the form actually secreted into the extracellular media as visualized by SDS-PAGE under reducing conditions. Under nonreducing conditions, this fibronectin component is secreted as a dimer (data not shown), but always with a monomer showing a molecular weight of 240 K. Results from this pulse-chase study correlate the acquisition of Endo H resistance of intracellular fibronectin seen in Figure 2 with that of the secretory form of fibronectin visualized in Figure 3, indicating that melanoma fibronectin is first processed by the Golgi apparatus prior to secretion.

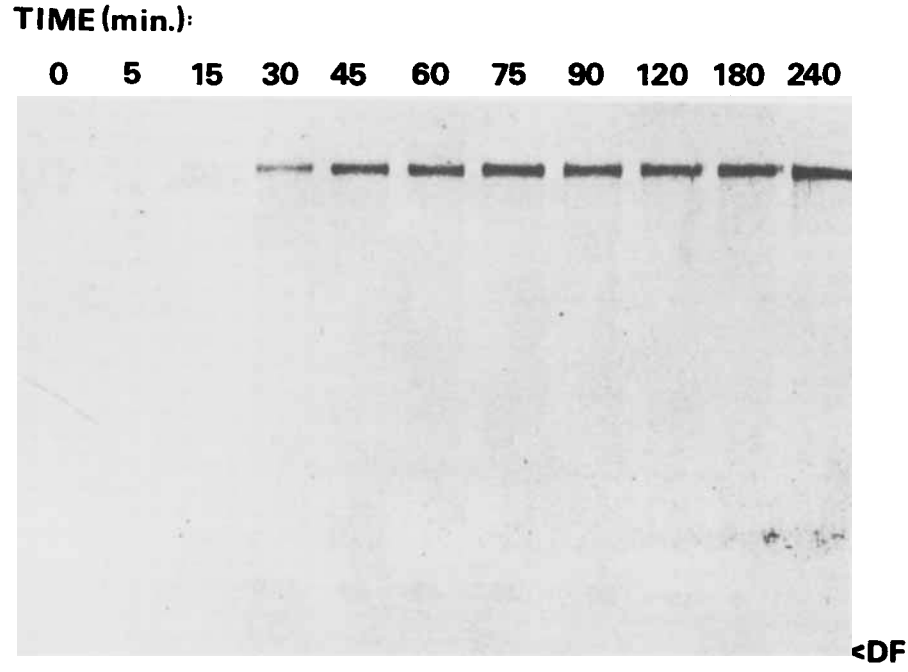

Fig. 3. Pulse-chase biosynthetic analysis of secreted fibronectin from M21 melanoma cells. A parallel analysis to that represented in Figure 2 examined the soluble fibronectin in the spent tissue culture medium by indirect immunoprecipitation analysis with antiserum 1450 R5 at the time points indicated in the chase period. This fluorograph represents a 96-hr exposure.

Data from further experiments examining the Endo H sensitivitiy of secreted fibronectin in pulse-chase and long-term intrinsic labeling studies reveal that all secreted fibronectin of melanoma cells is Endo H-resistant (data not shown). In addition, the observations illustrated in Figure 3 demonstrate that in this cell type, the Endo H-sensitive form of fibronectin visualized in Figure 2, from 30 min on throughout the case period, is not secreted and most likely represents an intracellular biosynthetic intermediate or a pool of fibronectin.

The next experiments were designed to examine the effects of monensin on secretion of fibronectin from melanoma cells because of the reported effects of this ionophore on the secretory and biosynthetic functions of the Golgi apparatus [19–22]. Figure 4 shows the results of a pulse-chase study of fibronectin secretion following a 2 hr exposure of melanoma cells to 10^{-7} M monensin. Figure 4A depicts control fibronectin secretion of these same cells, initially detectable at 20 min into the chase period. Secretion of the 240 K component continues to increase until 60 min into the chase, whereas at subsequent time points no further secretion of fibronectin was determined by soft laser densitometric scans of these fluorographs. In contrast to these results, preexposure of M21 melanoma cells to 10^{-7} M monensin results in a delay of 10–20 min in the first detectable secretion of fibronectin, with a more striking quantitative inhibition of secretion being observed in parallel, identical cultures (Fig. 4B). Densitometric scans of fluorographs resulting from these experiments verify this

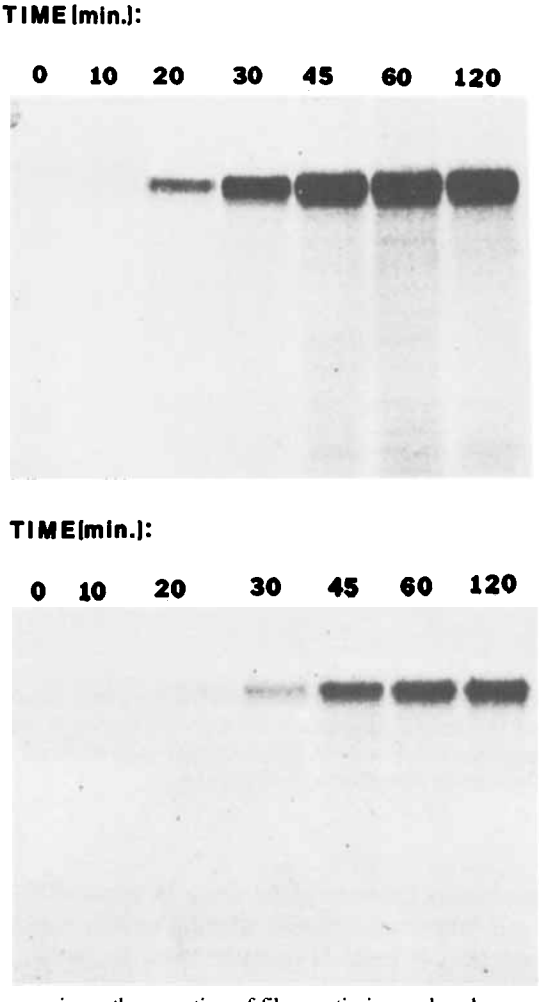

Fig. 4. Effect of monensin on the secretion of fibronectin in a pulse-chase analysis of M21 melanoma cells. A) Indirect immunoprecipitation profile of fibronectin with antiserum 1450 R5 at the time points indicated for control cell populations. B) An analysis done in parallel, following a 2-hr preexposure to 10^{-7} M monensin. These fluorographs represent parallel 96-hr exposures of 5% acrylamide SDS-PAGE analyses.

observation, indicating an overall 68.4% inhibition of fibronectin secretion at 120 min relative to secretion of control cells. In addition, close examination of the apparent molecular weight of fibronectin secreted from monensin-treated cells indicated that it was slightly lower. Thus, it seems that monensin may affect biosynthetic functions as well as intracellular transport activity of melanoma cells, an observation we have previously made in studies of a chondroitin sulphate-like proteoglycan associated with these cells [24].

Long-term labeling experiments (18 hr) with protein precursors showed an overall inhibition of fibronectin secretion from monensin-treated melanoma cells ranging from 20% to 35%. These data demonstrate that the monensin-treated cells,

displaying a 68.4% inhibition in fibronectin secretion after 2 hr in pulse-chase experiments (Fig. 4), continued to secrete fibronectin in long-term experiments to eventually approach the level of secretion of control cells. However, all immunoprecipitates analyzed from monensin-treated cells showed a lower apparent M_r of fibronectin than in control cells.

Initial investigations to determine the molecular basis for this effect specifically examined the biosynthetic incorporation of the oligosaccharide precursors ^{3}H-glucosamine, ^{3}H-galactose, and ^{3}H-fucose into secreted fibronectin in the presence of monensin in long-term (18 hr) labeling experiments. The results of such an experiment are shown in Figure 5. When ^{3}H-glucosamine was used as a glycoprotein precursor (Fig. 5, lanes A and B), we observed an apparent lower molecular weight for fibronectin secreted from monensin-treated cells (lane B); however, the relative amount of this component decreased by only 28% when judged by densitometric analysis. In contrast to these results, galactose incorporation (Fig. 5, lanes C and D) into fibronectin secreted in the presence of monensin was decreased 76% (lane D) versus control immunoprecipitates (lane C). Parallel experiments examining fucose incorporation (Fig. 5, lanes E and F) revealed a 48% decrease in fibronectin secreted from monensin-treated cells (lane F). These data stimulated investigations into the Endo H sensitivity of fibronectin synthesized by monensin-treated cells and initial 2 hr pulse-chase studies reveal that whereas control cells convert 75% of synthesized

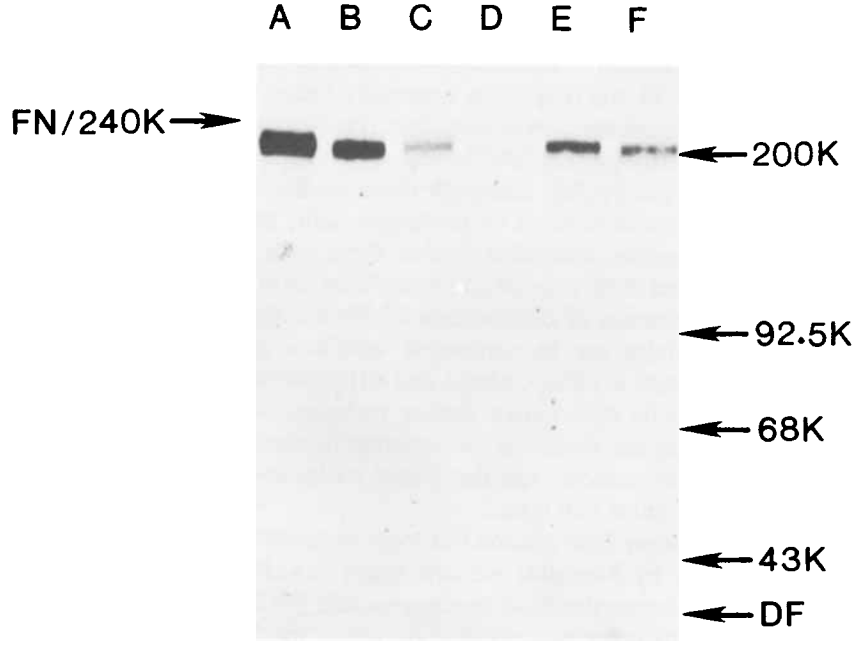

Fig. 5. Effects of monensin on the incorporation of oligosaccharide precursors into secreted fibronectin from M21 melanoma cells. Lanes A, C, and E represent control profiles of fibronectin, biosynthetically labeled with ^{3}H-glucosamine, ^{3}H-galactose, and ^{3}H-fucose, respectively. Lanes B, D, and F represent fibronectin profiles obtained in parallel experiments with M21 melanoma cells that were exposed to 10^{-7} M monensin for 18 hr and labeled with ^{3}H-glucosamine, ^{3}H-galacose, and ^{3}H-fucose, respectively. Migration of molecular weight standards is indicated in this 7-day exposure fluorograph.

fibronectin into an Endo H-resistant form by 120 min, fibronectins of monensin-treated cells are completely sensitive to Endo H degradation in this time period. Taken together, these results indicate that melanoma fibronectin can be intrinsically labeled with several oligosaccharide precursors, and suggest that monensin may have a differential effect on oligosaccharide biosynthesis involving fucose and galactose in terminal glycosylation reactions and glucosamine in glycosylation of core/high-mannose oligosaccharides as well as a potential effect on oligosaccharide processing.

DISCUSSION

In investigations described here, we have made use of a specific polyclonal antiserum to human plasma fibronectin to study the biosynthesis and secretion of a related cellular form of this molecule of human melanoma cell lines. The results indicate that melanoma cells contain fibronectin on their surface as determined by radioimmunometric binding analysis and secrete a fibronectin glycoprotein with an apparent monomer M_r of 240,000 (Table I, Fig. 1). These studies also demonstrate that the fibronectin glycoprotein of melanoma cells migrates considerably slower in SDS-PAGE with an apparent M_r of 240,000 in contrast to the M_r of 220,000 reported for plasma fibronectin.

Our studies on the biosynthesis of fibronectin in melanoma cells employed pulse-chase analysis of specific immunoprecipitates together with Endo H digestion (Fig. 2). The results indicated that fibronectin is initially synthesized as an Endo H-sensitive glycoprotein, suggesting that the earliest immunologically detectable fibronectin contains unprocessed N-linked oligosaccharides [22,29,30]. The acquisition of Endo H resistance at 30 min (Fig. 2) is kinetically linked with the first appearance of the secreted 240 K monomer, indicating that fibronectin of melanoma cells is first processed and terminally glycosylated by the biosynthetic processes that take place in the Golgi apparatus [22,29,30]. Although these studies indicate that 80% of newly synthesized fibronectin is secreted by melanoma cells, an Endo H-sensitive form of the glycoprotein remains detectable within these cells after 4 hr into the chase period, suggesting that there may be an intracellular form of fibronectin in melanoma cells. Our data on kinetics of biosynthesis of fibronectin in human melanoma cells and Endo H sensitivity are in agreement with two previous studies examining fibronectin biosynthesis in chick embryo and NIL8 hamster cells [31,32], suggesting that human tumor cells may utilize similar pathways for fibronectin biosynthesis. The key difference in our studies is the apparent intracellular maintenance of Endo-sensitive forms of fibronectin and the larger molecular weight of the fibronectin secreted by this malignant cell type.

Fibronectins larger than plasma fibronectins found in amniotic fluid have been described previously by Ruoslahti and colleagues as well as in a previous report that described sulfated fibronectins from melanoma cells [33,34]. Taken together with our results, these findings point to a possible oncofetal species of the fibronectin glycoprotein. Preliminary studies from our laboratory on strains of human fetal melanocytes indeed demonstrate that these cells secrete fibronectin molecule, indistinguishable in molecular form from that of human melanoma cells, again suggesting the existence of a possible oncofetal form of this glycoprotein (Bumol et al, in preparation).

Our studies investigating the effects of monensin on fibronectin biosynthesis and secretion demonstrated that the ionophore inhibits the secretion of this molecule

from melanoma cells as well as the incorporation of several oligosaccharide precursors such as fucose and galactose into this secreted glycoprotein (Figs. 4, 5). These results on a malignant cell type are partially in agreement with data from studies examining the effects of monensin on the secretion of fibronectin from normal human fibroblasts [19]; however, they differ with respect to fibronectin of melanoma cells appearing biosynthetically altered as it reveals an apparent lower M_r in the presence of monensin. In this regard, since monensin appears to disrupt the morphology and integrity of Golgi apparatus zones within the cell [20], it is possible that the primary effect of this ionophore on melanoma fibronectin biosynthesis is to inhibit the intracellular transport, oligosaccharide processing, and terminal glycosylation reactions associated with the Golgi apparatus. It will be necessary to verify this hypothesis by ultrastructural examinations of Golgi zones within monensin-treated melanoma cells and by biochemical analysis of glycopeptide structures on fibronectins isolated from melanoma cells before and after treatment with monensin. However, our results thus far demonstrate that the degree of glycosylation contributes considerably to the apparent molecular weight of fibronectin from melanoma cells when estimated by SDS-PAGE analysis of specific immunoprecipitates. The concept that glycosylation is a contributing factor in molecular-weight estimates was first suggested by analysis of the carbohydrate composition of the fibronectin of higher molecular weight found in amniotic fluid [33].

In conclusion, results from our studies indicate that the malignant melanoma cell synthesizes and secretes a biosynthetically altered fibronectin that exhibits higher molecular weight than normal plasma or cellular fibronectins. Cell-surface expression was noted, but recent immunofluorescence analysis of fibronectin topography on melanoma cells demonstrates the lack of organized fibrillar fibronectin networks and features a punctuated pericellular appearance of melanoma cell surface fibronectin (Bumol and Hayman, unpublished information). This surface expression of fibronectin could result from the transient expression of secreted fibronectin at the cell surface, and possibly suggests a functional role of this secreted adhesion-promoting molecule in the tumor biology of melanoma. Previous studies in B16 mouse melanoma have suggested that these fibronectin-negative cells can utilize endothelial cell fibronectin to mediate adhesion in an in vitro model for metastasis [35]. Our biosynthetic studies have shown that the bulk of fibronectin in human melanoma cells is secreted in soluble form (Fig. 3), and we have recently utilized this fact to isolate such molecules from the M14 melanoma cell line grown in chemically defined media. Preliminary investigations into functional properties of fibronectin produced by melanoma cells indicate that this glycoprotein can mediate attachament and spreading of such cells on various substrata, suggesting that the melanoma tumor cell can utilize its own biosynthetic product for phenomena relevant to its tumor biology. Additional studies will establish the specific roles that this glycoprotein may play in invasive and metastatic properties of human melanoma cells.

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REFERENCES

1. Pearlstein E, Gold L, Garcia-Pardo A: Mol Cell Biol 29:103, 1980.
2. Hynes RO: In Hay E (ed): "Cell Biology of Extracellular Matrix." New York and London: Plenum Press, 1981.
3. Yamada KM: In Hay E (ed): "Cell Biology of Extracellular Matrix." New York and London: Plenum Press, 1981.
4. Ruoslahti E, Hayman EG, Pierschbacher M, Engvall E: In Cunningham LW, Fredericksen DW (eds): "Methods in Enzymology, " Vol 82. New York: Academic Press, 1982, p 803.
5. Ruoslahti E, Engvall E: Biochim Biophys Acta 631:350, 1980.
6. Pierschbacher MD, Hayman EG, Ruoslahti E: Cell 26:259, 1981.
7. Ruoslahti E, Hayman EG, Engvall E: J Biol Chem 256:7277, 1981.
8. Wagner DD, Hynes RO: J Biol Chem 254:6746, 1979.
9. Hayashi M, Yamada KM: J Biol Chem 256:11292, 1981.
10. Sekiguchi K, Hakomori S: Proc Natl Acad Sci USA 77:2661, 1980.
11. Hayashi M, Schlesinger DH, Kennedy DW, Yamada KM: J Biol Chem 255:10017, 1980.
12. Hynes RO: Biochim Biophys Acta 458:73, 1976.
13. Yamada KM, Olden R: Nature (Lond) 275:179, 1978.
14. Vaheri A, Ruoslahti E: J Exp Med 142:530, 1975.
15. Hayman EG, Engvall E, Ruoslahti E: J Cell Biol 88:352, 1982.
16. Lloyd KO, Travassos LR, Takahashi T, Old LJ: J Natl Can Inst 63:623, 1979.
17. Guilano A, Irie RF, Morton DL, Rammin KP: Proc Am Assoc Cancer Res 19:133, 1978.
18. Chee DO, Boddie AW, Roth JA, Holmes EC, Morton D: Cancer Res 36:1503, 1976.
19. Uchida N, Smilowitz H, Tanzer ML: Proc Natl Acad Sci USA 76:1868, 1979.
20. Tartakoff A, Vassalli P: J Cell Biol 79:694, 1978.
21. John DC, Schlesinger MJ: Virology 103:407, 1980.
22. Strous GJ, Lodish HF: Cell 22:709, 1980.
23. Galloway DR, McCabe RP, Pellegrino MA, Ferrone S, Reisfeld RA: J Immunol 26:62, 1981.
24. Bumol TF, Reisfeld RA: Proc Natl Acad Sci USA 79:1245, 1982.
25. Tarentino AL, Trimble RB, Maley F: Meth Enzymol 5:574, 1980.
26. Bonner WM, Laskey RA: Eur J Biochem 46:83, 1974.
27. Morgan AC, Galloway DR, Wilson BS, Reisfeld RA: J Immunol Meth 39:233, 1980.
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
29. Fries E, Rothman JG: Proc Natl Acad Sci USA 77:3870, 1980.
30. Kornfeld R, Kornfeld S: In "The Biochemistry of Glycoproteins and Proteoglycans." New York and London: Plenum Press, Vol 1, 1980.
31. Olden K, Hunter VA, Yamada KM: Biochim Biophys Acta 632:408, 1980.
32. Choi MG, Hynes RO: J Biol Chem 254:12050, 1979.
33. Ruoslahti E, Engvall E, Hayman EG, Spiro RG: Biochem J 193:295, 1981.
34. Wilson BS, Ruberto G, Ferrone S: Biochem Biophys Res Commun 101:1047, 1981.
35. Nicolson GL, Irimura T, Gonzalez R, Ruoslahti E: Exp Cell Res 135:461, 1981.