Modulation of Lysosomal-associated Membrane Glycoproteins during Retinoic Acid-induced Embryonal Carcinoma Cell Differentiation*

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Differentiation of the murine embryonal carcinoma (EC) cell lines F-9 and PC-13, induced by β all-trans-retinoic acid (RA) resulted in an increased level of two lysosomal-associated membrane glycoproteins (LAMP-1 and LAMP-2). After differentiation, the levels of both LAMPS in the EC cells were comparable to those found in visceral and parietal endoderm cell lines (PSA-5E and PYS-2, respectively). RA treatment of the EC cells resulted in an increase in the apparent Mr of both LAMPS, apparently due to increased glycosylation because the deglycosylated LAMP-1 from undifferentiated and from differentiated cells had a similar electrophoretic migration. Indeed, the binding of 125I-labeled 1-phytohemagglutinin (L-PHA) to glycoproteins with Mr of 90,000–130,000 increased after differentiation and about 24 times more 125I-labeled L-PHA bound to LAMP-1 isolated by immunoprecipitation from extracts of RA-treated F-9 cells than to LAMP-1 from undifferentiated cells. The increased level of the LAMPS was detected in F-9 cells treated with >10−7 M RA and required >48 h of treatment as did the increased expression of the B1 chain of laminin, an established marker for differentiation in this system. LAMP-1 and L-PHA-reactive glycoproteins were localized by fluorescence technique to intracellular vesicles, presumably lysosomes, and to the cell surface and both increased after RA treatment. LAMP-2 was barely detectable intracellularly in undifferentiated cells but could be detected clearly after differentiation. In contrast, no LAMP-2 could be detected on the cell surface either before or after differentiation of F-9 cells. The increased level and glycosylation of both LAMP-1 and LAMP-2 was observed also in cells treated with a synthetic chalcone carboxylic acid analog of RA and by combination of either retinoid with dibutyryl cyclic AMP. These results demonstrate that differentiation of EC cells is accompanied by changes in the synthesis and glycosylation of LAMP glycoproteins and that these changes are specific for the cell type that results after differentiation.

Recently, two highly glycosylated glycoproteins with Mr of about 110,000 have been identified as major integral components of lysosomal membranes and designated lysosomal-associated membrane glycoproteins (LAMP-1 and LAMP-2)1 (1–6). These glycoproteins are also present at low levels in the plasma membrane of various cells, presumably because of membrane flow from lysosomes to the plasma membrane (3, 6). It has been suggested they might be involved in cell adhesion to extracellular matrix components and that the adhesion is inversely related to the extent of their glycosylation (7). The expression of these glycoproteins on the cell surface is modulated during cellular differentiation and malignant transformation (3, 8). The LAMPS are highly glycosylated glycoproteins carrying about 18 N-linked oligosaccharide side chains and several O-linked chains, and their Mr drops to 40,000–43,000 after deglycosylation (2, 4, 8). LAMP-1 has been identified as the major carrier of L-PHA-reactive oligosaccharides in a variety of cell types (9). The GlcNAcβ1-6Manα1-6Manβ8 branch of oligosaccharides has been shown to be the preferred site for the addition of polyactosaminoglycan chains, which are made up of repeating units of (Galβ1-3GlcNAcβ1-3). Alterations in branched N-linked oligosaccharide chains of cellular glycoproteins, specifically those carrying the β1-6-linked lactosamine antennae to which L-PHA binds, have been correlated with transformation and the acquisition of the metastatic phenotype (9).

Polylactosaminoglycans present on the surface of embryonic cells, including mouse embryonal carcinoma cells, have been shown to carry many developmentally expressed antigens including SSEA-1 (stage-specific embryonic antigen 1) (13), FH6 (14), and the blood group I, i antigens (13). The expression of many antigens, which are defined by carbohydrate epitopes, including those mentioned above, are modulated during RA-induced differentiation of murine embryonal carcinoma cells (15). Therefore, we examined the expression and glycosylation of both LAMP-1 and LAMP-2 during RA-induced differentiation of the murine embryonal carcinoma cell line F-9.

EXPERIMENTAL PROCEDURES

Materials—RA ((E)-3,7-dimethyl-9-(2,3,3-trimethyl-1-cyclohexene-1-yl)-2,4,6,8-nonatetraenoic acid) and Ch55 ((E)-4-[3-(3,5-ditertbutylphenyl)-3-oxo-1-propenyl]benzoic acid), a chalcone carboxylic acid derivative with retinoid like properties (16, 17), were a gift from BASF (Ludwigshafen, Federal Republic of Germany). N4P-Dibutyryl cyclic AMP, endoglycosidase H, and endo-β-galactosidase were purchased from Boehringer Mannheim. TranSMART label (>1,000 Ci/mmol), a mixture of 35S-labeled methionine and cysteine, was purchased from ICN. Goat anti-rat IgG, L-PHA, and the fluorescein isothiocyanate (FITC)-labeled derivative of L-PHA were obtained from Vector Laboratories (Burlingame, CA). The rat monoclonal

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1 The abbreviations used are: LAMP, lysosomal-associated membrane glycoprotein; BtCAMP, dibutyryl cyclic AMP; Ch55, (E)-4-[3-(3,5-ditertbutylphenyl)-3-oxo-1-propenyl]benzoic acid; FITC, fluorescein isothiocyanate; L-PHA, L-phytohemagglutinin, RA, retinoic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Me2SO, dimethyl sulfoxide; PBS, phosphate buffered saline.
antibodies against mouse LAMP-1 (1D4B) and LAMP-2 (ABL-70) were a generous gift from Dr. J. Thomas August (The Johns Hopkins University School of Medicine, Baltimore, MD). The sources of all other materials have been described previously (18).

**Cell Culture**—The murine embryonal carcinoma cell line F-9 (19) was a generous gift from Dr. J. Thomas August (Johns Hopkins Institute, Rehovot, Israel) while the murine embryonal carcinoma cell line PC-13 (19), the murine parietal endodermal cell line PYS-2 (20), and the murine visceral endodermal cell line PSA-5E (21) were obtained from Dr. Angie Rizzino (University of Nebraska, Lincoln, NE). All cells were routinely grown in a 1:1 (v/v) mixture of Ham's F12 and Dulbecco's modified 1:1000 supplement with 10% fetal bovine serum (Hazelton, Lenexa, KS) and antibiotics (penicillin and streptomycin, both at 100 μg/ml). They were induced to differentiate by diluting RA or Ch55 from a 10 mM stock solution in the tissue culture medium.

**Type** has been described previously (1). The sources of all enzymes when F-9 embryonal carcinoma cells were induced to differentiate by RA into primitive endoderm-like cells, the amount of LAMP-1 increased, as determined by immunoblotting with specific antibodies against LAMP-1. In addition, LAMP-1 mobility decreased, which indicated increased glycosylation (Fig. 1, panel I, lanes A and B). Likewise, RA treatment of PC-13 cells, which differentiated into visceral endoderm-like cells, resulted in an increase in the expression and decreased mobility of LAMP-1 (Fig. 1, panel I, lanes C and D). Both differentiated cell lines representing visceral endoderm (PSA-5E) and parietal endoderm (PYS-2) cells, which were refractory to RA treatment as far as growth.

**RESULTS**

**Modulation of Expression and Glycosylation of LAMPS in Embryonal Carcinoma Cells and Their Differentiated Counterparts**—When F-9 embryonal carcinoma cells were induced to differentiate by RA into primitive endoderm-like cells, the amount of LAMP-1 increased, as determined by immunoblotting with specific antibodies against LAMP-1. In addition, LAMP-1 mobility decreased, which indicated increased glycosylation (Fig. 1, panel I, lanes A and B). Likewise, RA treatment of PC-13 cells, which differentiated into visceral endoderm-like cells, resulted in an increase in the expression and decreased mobility of LAMP-1 (Fig. 1, panel I, lanes C and D). Both differentiated cell lines representing visceral endoderm (PSA-5E) and parietal endoderm (PYS-2) cells, which were refractory to RA treatment as far as growth.

**Fig. 1.** Modulation of expression and glycosylation of LAMPS in embryonal carcinoma cells and their differentiated counterparts. Two embryonal carcinoma cell lines F-9 (Lanes A and B) and PC-13 (Lanes C and D) and the visceral endodermal cell line, PSA-5E (Lanes E and F), and PYS-2, a parietal endodermal cell line (Lanes G and H) were grown in the medium containing 0.01% MeSO (-) or in the presence of 1 × 10^{-5} M RA (+) for 5 days. Equal amounts of detergent-soluble cellular proteins were separated by SDS-PAGE and transferred to nitrocellulose sheets. The nitrocellulose sheets were treated as follows. Panel I, the nitrocellulose sheet was incubated with agitation for 2 h at 23 °C with anti-LAMP-1 (1D4B hybridoma supernatant diluted 1:10 in blocking buffer). The nitrocellulose sheet was then washed and processed as described under "Experimental Procedures." The dried nitrocellulose sheet was exposed to film for 3 days in the presence of an intensifying screen. Panel II, the nitrocellulose sheet was incubated with anti-LAMP-2 (ABL-93) hybridoma supernatant diluted 1:10 as described above for anti-LAMP-1. The nitrocellulose sheet was exposed to film for 6 days in the presence of an intensifying screen. Panel III, the nitrocellulose sheet was blocked as described above for the antibodies. The sheet was then incubated with 125I-labeled L-PHA (4 × 10^{5} cpm/blot) for 2 h at 23 °C with agitation. Following extensive washing the nitrocellulose sheet was dried and exposed to film for 2 days in the presence of an intensifying screen. The numbers on the right indicate the migration of the prestained molecular weight standards α-macroglobulin (M, 180,000), β-galactosidase (M, 116,000), fructose-6-phosphate kinase (M, 84,000), pyruvate kinase (M, 58,000), and fumarase (M, 48,000).
and morphology were concerned, did not show any significant differences in the amount or mobility of LAMP-1 after RA treatment (Fig. 1, panel I, lanes E, F, G, and H). However, the intrinsic mobility of LAMP-1 from the PYS-2 cells was lower than that from the PSA-5E cells (Fig. 1, panel I, lanes E and G).

LAMP-2 was usually undetectable in the undifferentiated F-9 and PC-13 embryonal carcinoma cells (Fig. 1, panel II, lanes A and C). However, RA-induced differentiation of both cell lines resulted in a marked increase in the expression of LAMP-2 (Fig. 1, panel II, lanes A, B, C, and D). Both untreated PSA-5E and PYS-2 cells expressed high levels of LAMP-2, which was not affected by RA treatment (Fig. 1, panel II, lanes E, F, G, and H). As with LAMP-1, the mobility of LAMP-2 from the PYS-2 cells was lower than that from the PSA-5E cells.

The decrease in the mobility of LAMPS after RA-induced differentiation of F-9 and PC-13 cells and the difference in mobility between the PYS-2 and PSA-5E cells suggest that they differ in the degree of glycosylation. Therefore, the same samples were analyzed for binding of L-PHA. Fig. 1 (panel III) shows that differentiation of both F-9 and PC-13 cells was accompanied by a dramatic increase in the ability of 125I-labeled L-PHA to bind to glycoproteins after separation by SDS-PAGE. The major glycoproteins of 90-130 kDa are presumably LAMP-1 and LAMP-2 (Fig. 1, panel III, lanes A and B). Although RA treatment had no effect on the growth of morphology of PSA-5E cells, it did result in an increased binding of L-PHA to glycoproteins of >150 kDa (Fig. 1, panel III, lanes E and F). PYS-2 cells had the highest levels of L-PHA-reactive oligosaccharides, of which the major ones were glycoproteins of 110 and 48 kDa. The pattern of L-PHA binding to PYS-2 glycoproteins was similar to that of RA-induced F-9 glycoproteins (Fig. 1, compare lanes B and G in panel III). RA treatment had no effect on the L-PHA-reactive glycoproteins of the PYS-2 cells (Fig. 1, panel III, lanes G and H).

RA Dose-dependent Modulation of LAMP-1 and LAMP-2 in F-9 Embryonal Carcinoma Cells—RA treatment resulted in a dose-dependent increase in the level of both LAMP-1 and LAMP-2 but showed different dose-response relationships (Fig. 2). Both LAMP-1 and LAMP-2 were maximally increased (2.5- and 8-fold, respectively) after a 5-day treatment with 10^{-6} M RA. The increase in LAMP-1 level and the decrease in its mobility were detectable after treatment with RA at doses as low as 10^{-9} M. In contrast, marked increases in LAMP-2 were detected only at higher concentrations of RA > 10^{-7} M (Fig. 2). It is noteworthy that the optimal increase in the levels of both LAMPS occurred at 10^{-6} M and declined after treatment with 10^{-5} M RA. The reason for this decline is not known.

RA Dose-dependent Increase in 125I-labeled L-PHA Binding to Glycoproteins of F-9 Embryonal Carcinoma Cells—RA treatment of F-9 embryonal carcinoma cells resulted in a dose-dependent increase in the binding of 125I-labeled L-PHA to glycoproteins of 90-130 kDa after separation by SDS-PAGE. As can be seen in Fig. 2 the maximal increase in L-PHA binding was first detectable in cells treated with 10^{-9} M RA and increased especially at the higher RA concentrations (10^{-5} and 10^{-4} M). In this experiment we used the polyacrylamide gels for 125I-labeled L-PHA binding rather than transfer the glycoproteins onto nitrocellulose membranes as in Fig. 1 (panel III). This is probably the reason for the decrease in the labeling of glycoproteins of <84 kDa on the gel compared with the nitrocellulose.

Following immunoprecipitation, equal amounts of LAMP-
Lysosomal Glycoprotein Modulation during Differentiation compared with LAMP-1 derived from controls. This provided direct evidence that the glycosylation of LAMP-1 is modulated during RA-induced differentiation. Attempts to immunoprecipitate LAMP-2 from treated or untreated cells were unsuccessful because the amount or the labeling of this glycoprotein with [³⁵S]methionine was much lower than that of LAMP-1. These results do not exclude the possibility that in RA-treated cells there are ϕ-PHA-binding proteins with M₉ of about 116,000 that are distinct from LAMPS.

Kinetics of the RA-induced Increase in Expression of LAMP-1 and LAMP-2—RA-induced differentiation resulted in a time-dependent increase in the expression and decreased mobility of both LAMP-1 and LAMP-2 (Figs. 4 and 5). The amount of LAMP-1 began to increase after 72 h of treatment with 10⁻⁶ M RA (Figs. 4 and 5, upper panels). The induction of LAMP-2 followed the same pattern as LAMP-1. The level of LAMP-2 in control cultures increased somewhat with time, although this observation was variable, possibly due to the presence of some spontaneously differentiated cells in some of the untreated cultures (Figs. 4 and 5, middle panels). The increased levels of both of these glycoproteins exhibited the same kinetics as the increase in the expression of the B1 chain of laminin, which is an established marker for differentiation in this system (26) up to 96 h of treatment. However, between 96 and 120 h, the amount of cell-associated laminin decreased (Figs. 4 and 5, lower panels), probably due to secretion and deposition of laminin in the extracellular compartment evidenced by immunofluorescence microscopy (data not shown), whereas the amount of LAMPS remained constant between 96 and 120 h.

Localization of LAMP-1, LAMP-2 and ϕ-PHA Receptors in F-9 Cells—The majority of LAMP-1 was detected in permeabilized F-9 cells in association with lysosomes (Fig. 6A), and a punctate pattern of labeling was detected on the surface of nonpermeabilized cells (Fig. 7A). RA-induced differentiation increased the labeling intensity of LAMP-1 in the lysosomes as well as on the cell surface (Fig. 7A). LAMP-2 was detected in a very low level in permeabilized control cultures (Fig. 6C), but after differentiation its level increased markedly and it was also associated with the lysosomes (Fig. 6D). LAMP-2 could not be detected on the cell surface of either the control cells or the differentiated cells (data not shown). Binding of FITC-labeled ϕ-PHA was detected in undifferentiated and differentiated permeabilized cells associated with the cell membrane as well as with intracellular vesicles (Fig.

![Fig. 4. Kinetics of RA-induced increased expression of LAMPS and laminin in F-9 cells. Cells were treated with 1 x 10⁻⁶ M RA (+) for the indicated times while the control cultures (−) were treated with 0.01% Me₂SO. The cells were harvested and processed for immunoblotting as described in Fig. 1. The figure is a composite of three separate blots. The top blot was probed with anti-LAMP-1 and was exposed for 3 days. The middle blot was probed with anti-LAMP-2 and was exposed for 6 days. The bottom blot was probed with a 1:200 dilution of a polyclonal antibody against murine laminin and was exposed for 3 days.](http://www.jbc.org/)

![Fig. 5. Quantitation of the increased expression of LAMP-1, LAMP-2, and laminin during RA-induced differentiation of F-9 cells. The x-ray films from Fig. 4 were analyzed by densitometric scanning.](http://www.jbc.org/)

![Fig. 6. Localization of LAMPS and ϕ-PHA-reactive glycoproteins within F-9 cells. Cells were grown in the presence of 0.01% Me₂SO (A, C, and E) or in the presence of 1 x 10⁻⁶ M RA (B, D, and F) for 5 days. On day 3 the cells were subcultured onto glass coverslip. The cells were fixed, permeabilized, and stained with anti-LAMP-1 (A and B), anti-LAMP-2 (C and D), or FITC-labeled ϕ-PHA (E and F) as described under "Experimental Procedures." The cells were then analyzed by indirect immunofluorescence. Representative fields are presented (× 400).](http://www.jbc.org/)
when cells were pulse-labeled for 5 min and harvested immediately, a precursor of M, of approximately 98,000 was immunoprecipitated with anti-LAMP-1 antibodies (Fig. 8A). This precursor was sensitive to endoglycosidase H and was hydrolyzed to a M, 43,000 core polypeptide (Fig. 8B). There was no difference in the migration of the endoglycosidase H susceptible form or the polypeptide core of LAMP-1 from control and RA-treated cells (Fig. 8, A and B), which suggests that the altered migration of the mature molecules (Fig. 8C) is due to a posttranslational modification, presumably glycosylation via addition of GcNAcα1-6 branches. These results are in excellent agreement with the reports by D’Souza and August (23) that LAMP-1 is synthesized as a core polypeptide of M, 43,000 and is posttranslationally modified by glycosylation to a mature form of M, 110,000 (23). The apparent M, of the core polypeptide is similar to the predicted size based on the nucleotide sequence for the murine cDNA (27, 28).

Increased Binding of 125I-labeled L-PHA to Glycoproteins of M, 90-130 kDa after Differentiation of Murine Embryonal Carcinoma Cells—Previous studies have demonstrated that RA treatment of murine F-9 embryonal carcinoma cells results in the appearance of cells with properties of primitive endoderm, while RA in combination with Bt2cAMP results in the appearance of parietal endoderm (29, 30). Treatment with Bt2cAMP alone had no effect on the differentiation of these cells (30). RA-induced differentiation resulted in an increased binding of 125I-labeled L-PHA to glycoproteins of M, 90-130 kDa after separation by SDS-PAGE (Fig. 9, left panel, lanes A and B). Treatment of F-9 cells with RA in combination with Bt2cAMP also resulted in an increase in the binding of L-PHA as well as in a decrease in electrophoretic mobility, apparently due to an increase in glycosylation of the glycoproteins (Fig. 9, left panel, lane C). Ch55 treatment alone or in combination with Bt2cAMP did not result in changes in electrophoretic mobility (data not shown). Resistance to the enzyme may result from modifications of the poly glutaminyl oligosaccharide chains such as sulfation of Gal or αL-3 fucosylation of GcNAc residues (13).

Enzymatic Digestion of LAMP-1 Reveals That Altered Mobility Is Due to Increased Glycosylation—To determine whether the decreased mobility of LAMP-1 derived from RA-treated cells was due to an increase in glycosylation or whether it was due to some alteration in the polypeptide backbone, LAMP-1 immunoprecipitated from both control and RA-treated cells was treated with endoglycosidase H, which cleaves high mannose-type oligosaccharide side chains but leaves complex-type chains intact. When control and RA-treated cells were labeled overnight (steady state) with Trans35S-label and LAMP-1 was immunoprecipitated from the RA-treated cells, it migrated slower on SDS-PAGE compared with the control form (Fig. 8C) and was insensitive to endoglycosidase H digestion (data not shown). In contrast, LAMP-1 expression increased with all treatments that induced differentiation (Fig. 9, middle panel, lanes A-F). As with the L-PHA binding, treatment with RA or Ch55 in

[Figure 7: Localization of LAMP-1 and L-PHA-reactive glycoproteins on the surface of F-9 cells. Cells were grown in medium containing 0.01% Me2SO or in the presence of 1 x 10^{-7} M RA (B and D) for 5 days. Prior to labeling with Trans35S-label, the cells were incubated in methionine-free medium for 4 h. The samples in the A and B lanes were metabolically labeled for 5 min (100 μCi/ml) and harvested and solubilized immediately. The samples in the C lanes were labeled overnight (100 μCi/ml) in methionine-free medium. LAMP-1 was immunoprecipitated from cell extracts containing 1 x 10^{10} trichloroacetic acid-precipitable counts per min as described under "Experimental Procedures." The immunoprecipitates in A lanes were incubated in the enzyme digestion buffer for 16 h at 37°C. The samples in B lanes were incubated in the same buffer containing 5 units of endoglycosidase H under the same conditions.

[Figure 8: Endoglycosidase digestion of LAMP-1. F-9 cells were grown in medium containing 0.01% Me2SO or in the presence of 1 x 10^{-7} M RA (+) for 5 days. Prior to labeling with Trans35S-label, the cells were incubated in methionine-free medium for 4 h. The samples in the A and B lanes were metabolically labeled for 5 min (100 μCi/ml) and harvested and solubilized immediately. The samples in the C lanes were labeled overnight (100 μCi/ml) in methionine-free medium. LAMP-1 was immunoprecipitated from cell extracts containing 1 x 10^{10} trichloroacetic acid-precipitable counts per min as described under "Experimental Procedures." The immunoprecipitates in A lanes were incubated in the enzyme digestion buffer for 16 h at 37°C. The samples in B lanes were incubated in the same buffer containing 5 units of endoglycosidase H under the same conditions. The samples in C lanes were immediately dissolved in SDS-PAGE sample buffer and stored at -80°C until analysis.

[Figure 9: Effect of other differentiation inducing agents on the expression of LAMPs and L-PHA-reactive glycoproteins. F-9 cells were grown in medium containing: Lane A, 0.01% Me2SO; lane B, 1 x 10^{-7} M RA; lane C, 1 x 10^{-7} M RA + 1 mM Bt2cAMP; lane D, 1 x 10^{-7} M Ch55; lane E, 1 x 10^{-7} M Ch55 + 1 mM Bt2cAMP; lane F, 1 mM Bt2cAMP for 5 days. The expression of L-PHA-reactive glycoproteins was analyzed by incubating 125I-labeled L-PHA with the separated cellular proteins immobilized on nitrocellulose as in Fig. 1. The expression of both LAMP-1 and LAMP-2 were analyzed as in Fig. 1.]
combination with BtcAMP resulted in a slight decrease in the mobility of the molecule when compared to either compound alone. LAMP-2 was barely detectable in undifferentiated cells; however, differentiation induced by either RA, Ch55, or the combinations of each of these agents with BtcAMP was accompanied by a large increase in the level of LAMP-2. The mobility of LAMP-2 after treatment with RA or Ch55 in combination with BtcAMP decreased in comparison with LAMP-2 from cells treated with RA or Ch55 alone (Fig. 9, right panel, compare lanes B and D to C and E). These changes in mobility suggest that differentiation along the parietal endodermal differentiation pathway, induced by treatment with RA or Ch55 in combination with BtcAMP, involves greater increases in glycosylation of LAMP-1 and LAMP-2 than differentiation to primitive endoderm induced by treatment without BtcAMP.

**DISCUSSION**

This study has demonstrated that RA-induced differentiation of F-9 embryonal carcinoma cells is accompanied by a time- and dose-dependent increase in the expression of both LAMP-1 and LAMP-2. The increase in LAMP-1 level was accompanied by a dramatic increase in the ability of L-PHA to bind to it, which is indicative of increased GlcNAcβ1-6 branching (31). In addition, the cell surface expression of L-PHA receptors and LAMP-1 increased after differentiation. The increased level of both LAMP-1 and LAMP-2 had the same kinetics as the increase of the laminin B1 chain, indicating that the increased expression of the LAMPS may be considered as a marker for endodermal differentiation in the embryonal carcinoma cells. The increases in LAMPS may be associated with early stages of endodermal differentiation, since they were observed in F-9 cells induced to differentiate along distinct endodermal differentiation pathways directed by treatment with RA alone or in combination with BtcAMP. This conclusion is also supported by the increase in LAMPS in both F-9 and PC13 embryonal carcinoma cells, which are induced by RA to differentiate along distinct pathways, and by the constitutive expression of LAMPS in untreated P5E and PYS-2 cells, which represent visceral and parietal endodermal cells, respectively.

The distinct mobility of both LAMPS derived from the visceral endoderm P5E and the parietal endoderm PYS-2 on SDS-PAGE indicates that they differ in the extent of their glycosylation and that this property may be characteristic of the specific endodermal cell type. Further evidence to support this conclusion is drawn from the mobility of both LAMPS when the cells were treated with RA or Ch55 alone, which results in the formation of primitive endoderm compared with when they were treated with either compound in combination with BtcAMP, which results in the formation of parietal endoderm. These results would indicate that the increased glycosylation of both LAMPS may be a marker for the transition from primitive endoderm to parietal endoderm. The fact that Ch55 induced identical changes to those induced by RA indicates that the increased expression of these molecules is a result of the cell type induced rather than the differentiation-inducing agent and that the effects of retinoids on LAMP synthesis and glycosylation are not dependent on interaction with the cytosolic retinoic acid-binding protein because Ch55 does not bind to this protein (17). However, both retinoids bind to nuclear RA receptors, which belong to the steroid/thyroid hormone receptor family and act as ligand-activated trans-acting transcription-enhancing factors (32, 33). Recently, a direct role for the RA receptors in the regulation of transcription of laminin B1 chain was demonstrated in F-9 cells (34) and it is possible that these RA receptors are also involved in the increased expression of LAMPS in these cells. The increased glycosylation of LAMPS could also be mediated via increased gene expression, that of the GlcNAc transferase V.

Both LAMP-1 and LAMP-2 have been localized predominantly to the lysosomes in other cell types (1, 2), but a small percentage (2-5%) of both glycoproteins have been localized to the cell surface by indirect immunofluorescence (3, 6, 28). The cell surface expression of both LAMP-1 and LAMP-2 has been shown to decrease after 12-O-tetradecanoylphorbol myristate 13-acetate-induced differentiation of two leukemia cell lines, U937 and HL-60, into macrophage-like cells (3). Youakim et al. (35) found a decrease in the ability of Datura stramonium agglutinin to bind to LAMP-1 after spontaneous differentiation of CaCo-2 human colon carcinoma cells, suggesting that modification of the oligosaccharide side chain accompanies differentiation of those cells. These results are distinct from our observation with the embryonal carcinoma cells and may reflect the differences in the differentiation programs and possibly in the as yet undetermined function of the LAMPS on the cell surface.

Increased β1-6 branching, as measured by L-PHA binding, has been shown to be oncodevelopmentally regulated in both murine and human systems (8, 36). In the murine system, increased binding of L-PHA to LAMP-1 was correlated with increased metastatic potential (9). An increased cell surface expression of β1-6-branched oligosaccharides was specifically associated with increased invasiveness through an amniotic basement membrane in a murine tumor model system (37). Analysis of expression of the mRNA for LAMP-1 or P2B, as it was termed, revealed that there was no difference between nonmetastatic and metastatic murine tumor cells (28). These results support the hypothesis that increased metastatic potential is correlated with increased glycosylation and not with increased expression of the LAMP-1 polypeptide itself. If these conclusions can be extrapolated to the F-9 cells, then one would expect that RA-induced differentiation should increase the cells’ metastatic potential. Although we have not performed any in vivo experiments, Terrana et al. (38) reported that RA-induced differentiation of F-9 cells altered their organ preference from liver to lung after injection into the tail vein of mice. It is not clear whether changes in LAMP-1 could be responsible for this altered metastatic behavior.

Embryonal carcinoma cells are the most useful model for mammalian development (39). Undifferentiated embryonal carcinoma cells are analogous to the inner cell mass of the mouse embryo, while the primitive endodermal cells appear to separate the inner cell mass from the blastocyst. These cells are extraembryonic and are present only during a finite stage of embryogenesis but may play an inductive role in continued morphogenesis. The primitive endodermal cells that result after RA treatment of embryonal carcinoma cells possess many properties in common with metastatic tumor cells, including increased production of Type IV collagenase and plasminogen activator (29, 40). Increased production of both of these molecules like the increased β1-6 branching have been correlated with increased invasiveness in metastatic tumor models (41). An increase in invasiveness may be essential during embryo remodeling. Whether increased β1-6 branching on LAMP-1 plays a direct role in this phenomenon remains to be determined.

While much is known about the structure and expression of LAMP-1 and LAMP-2, little is known about their functions. The ubiquitous expression of LAMP-1 and the modulation of both molecules during differentiation and malignant
transformation would suggest an essential function. Laférté and Dennis (7) have demonstrated that purified LAMP-1 bound to extracellular matrix components, such as fibronectin, laminin, and Type IV collagen in solution. The ability of LAMP-1 to bind to these components was inversely related to the degree of its glycosylation, and it was suggested that the increased β1-6 branching of the molecule contributed to the increased invasive phenotype by decreasing adhesiveness (7). Interestingly, Tienari et al. (42) have found that RA-induced differentiation is accompanied by a decreased attachment of F-9 cells to both fibronectin and laminin. We have confirmed this observation with the F-9 cells used in our studies. This could be due to an increased glycosylation of the LAMP glycoproteins, which supposedly would reduce their adhesion to the extracellular matrix components.

Carlosson et al. (2) have suggested that either or both LAMPs could serve as architectural molecules in the lysosomes and that their heavy glycosylation protects them from proteolysis. Alternatively, Carlsson and Fukuda (43) suggested that the heavily glycosylated LAMP-1 may serve as an acceptor for microbial lectins. We have recently reported that RA-induced differentiation of F-9 embryonal carcinoma cells is accompanied by a 3-fold increase in the expression of the endogenous 14.5-kDa β-galactoside-binding lectin (44). The kinetics of the increased expression of the 14.5-kDa lectin, increased cell surface expression, and the dose-response relationship paralleled those for the increased expression of LAMP-1. In addition to binding terminal galactose residues, the 14.5-kDa lectin isolated from rat lung and calf heart has been shown to bind to polylactosamine oligosaccharides (45, 46). These results make LAMP-1 an excellent potential acceptor for the endogenous lectin.

In conclusion, in this study we have demonstrated that increased expression of both LAMP-1 and LAMP-2 accompanies differentiation of embryonal carcinoma cells. We have also established that differentiation results in increased glycosylation of LAMP-1.

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Modulation of lysosomal-associated membrane glycoproteins during retinoic acid-induced embryonal carcinoma cell differentiation.
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