Accumulation of Glucosylceramides in Multidrug-resistant Cancer Cells

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Multidrug-resistant (MDR) tumors and cancer cell lines demonstrate a wide variety of biochemical changes. In this study we used drug-sensitive wild-type (wt) cancer cell lines and respective MDR subclones, and we demonstrate the accumulation of distinct lipids in MDR cells. These lipids were either absent or present at very low levels in drug-sensitive cells. The compounds, termed lipid-1 and lipid-2, migrated on thin-layer chromatography as a doublet. They could be radiolabeled by incubating MCF-7-AdrR (Adriamycin-resistant) breast cancer cells with \(^{3}H\)serine, \(^{3}H\)palmitic acid, or \(^{3}H\)galactose. Utilization of these precursors by MCF-7-wt cells for synthesis of lipid-1 and -2 was minimal. Two inhibitors of sphingolipid biosynthesis, L-cycloserine and fumonisin B\(_{1}\), prevented the observed accumulation of the lipid compounds. An inhibitor of glucosylceramide synthesis, 1-phenyl-2-palmitylaminol-3-morpholino-1-propanol, completely abolished the formation of lipid-1 and -2 in MCF-7-AdrR cells and, to a lesser extent, inhibited the formation of lactosylceramides and gangliosides. Utilizing mass spectrometry, the multidrug resistance-associated lipids were further characterized as monoglycosylceramides of two major species that contained either 16-carbon (palmitic) or 24-carbon (linoceric and nervonic) fatty acids. The carbohydrate head group of glycolcosylceramides was identified as glucose, not galactose, thus designating the accumulated lipids as glucosylceramides. Incorporation of \(^{1}H\)palmitic acid into glucosylceramide was strikingly higher (8–10 times) in MCF-7-AdrR cells compared with MCF-7-wt cells. Since the rate of glucosylceramide degradation in MCF-7-AdrR cells was not attenuated, accelerated glycosphingolipid synthesis in MDR cells is suggested. Glucosylceramide also accumulated in KB-V-1, a vinblastine-resistant epidermoid carcinoma but not in KB-3-1, drug-sensitive wt cells. MDR ovarian adenocarcinoma cells (NIH-OVCAR-3) also contained elevated levels of glucosylceramide. Our results demonstrate a correlation between cellular drug resistance and alterations in glucosylceramide metabolism.

The multidrug-resistant (MDR) phenotype in its natural (inherited) or acquired form expresses resistance to a variety of drugs (1, 2). Current evidence suggests that this resistance is due to the ability of cells to lower intracellular drug concentration. Overexpression of the membrane efflux transporter P-gp is the most consistent alteration in MDR cells (1–4); however, the physiologic function and mechanisms of action of P-gp are largely unknown. Moreover, the widespread drug resistance of human lung tumors (5) is unrelated to overexpression of P-gp and indicates the existence of additional resistance mechanisms. The multifactorial nature of multidrug resistance is exemplified by a wide array of other biochemical changes including alterations in membrane fluidity and structure (1), elevated glutathione S-transferase activity (1, 6), down-regulation of topoisomerase II (6), increased phospholipase D activity (7), and elevated transcription of c-fos, c-myc and c-Ha-ras (4, 6, 8).

In this study, we show that a unique glycosphingolipid pattern is associated with MDR cells. Glycosphingolipid biosynthesis is initiated with formation of 3-ketosphinganine by condensation of serine and palmitoyl-CoA, followed by reduction of the keto group (producing sphinganine) and addition of an amide-linked fatty acid (Fig. 1A; Ref. 9). The ceramide formed is further metabolized to sphingomyelin or glycosylceramide by addition of the appropriate headgroup. De novo synthesis can be followed by incubation of cells with radiolabeled serine or palmitic acid and, in the case of glycosphingolipids, by incubation with radiolabeled galactose. In recent years, clues regarding the function of sphingolipids have been revealed by the discovery and use of sphingolipid biosynthesis inhibitors (Fig. 1A). These include inhibitors of 3-ketosphinganine synthase (L-cycloserine), ceramide synthase (FBO\(_{1}\)), and glucosylceramide synthase (PPMP) (10–13).

Glycosylceramides are the most widely distributed glycosphingolipids in cells serving as precursors for the biosynthesis of over 200 known glycosphingolipids. In addition to their role as building blocks of biological membranes, glycosphingolipids have long attracted attention because of their putative involvement in cell proliferation (14), differentiation (15, 16), and oncogenic transformation (17, 18). In addition, some metabolites of glycosphingolipids, such as sphingoid bases, ceramides, and lysosphingolipids, are suggested to have a second messenger function in signal transduction pathways involving growth (14, 19), apoptosis (20), and the action of tumor necrosis fac-

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1 The abbreviations used are: MDR, multidrug-resistant; AdrR, Adriamycin (doxorubicin)-resistant; BSA, bovine serum albumin; FAB/ MS, fast-atom bombardment/mass spectrometry; FB\(_{1}\), fumonisin B\(_{1}\); FBS, fetal bovine serum; Lac-cer, lactosylceramide; PC, phosphatidylcholine; PE, phosphatidylethanolamine; P-gp, P-glycoprotein; PPMP, 1-phenyl-2-palmitoylaminol-3-morpholino-1-propanol; SM, sphingomyelin; wt, wild-type.

2 Throughout this article "glycosylceramide" is used to refer to ceramide that bears nonspecified sugar moiety (either glucose or galactose).
cercells. This finding poses intriguing questions regarding the route of glucosylceramide formation. Inhibitors used in this study are listed in Table I. A. We now demonstrate that specific glycosphinogolipids, identified as glucosylceramides, accumulate in MDR cancer cells. This finding poses intriguing questions regarding the role of glucosylceramide in multidrug resistance.

**EXPERIMENTAL PROCEDURES**

**Materials—**Sphinganine, SM, and ceramides were purchased from Matreya, Inc. (Pleasant Gap, PA). ENHANCE, [3H]-serine (2.1 Ci/mmol), [3H]-palmitic acid (56.5 Ci/mmol), and [3H]-galactose (29.5 Ci/mmol) were purchased from DuPont NEN. Liquid scintillation mixture (Ecolume) was purchased from DuPont NEN. Rat liver microsomes from Dr. Merrill E. Goldsmith, National Cancer Institute. Rat liver macrophages were from Dr. Michael M. Gottesman, National Cancer Institute. All other biochemicals were from Sigma.

Cell Culture—MCF-7-wt and MCF-7-AdrR (Adriamycin-resistant) cells were kindly provided by Dr. Kenneth H. Cowan and Dr. Merrill E. Goldsmith, National Cancer Institute. Cells were maintained in RPMI 1640 medium containing 10% FBS (Bey), penicillin, streptomycin, L-glutamine, and sodium bicarbonate. MCF-7 cells, MDA-MB-231 cells, and KB-3–1 cells had been pre-run in 2% boxide in medium/water (1:1) and heated at 110°C prior to use (23).

Radiolabeling Using Sphingolipid Precursors—MCF-7-wt and MCF-7-AdrR cells were incubated for 24 h in medium containing [3H]-serine, [3H]-palmitic acid, or [3H]-galactose. The incorporation of radioactivity into cell lipids was assessed by autoradiography. Glucosylceramide accumulation in MDR cells is a new finding and more studies are needed to understand its role in drug resistance.
tively) and SM (6.9 and 10.9%, respectively). However, the incorporation of [3H]serine into lipid-1 and -2 was much more marked in MCF-7-AdrR cells (Fig. 3A), where it accounted for 4.5% of total radiolabeled lipids compared with 0.52% in MCF-7-wt cells. [3H]Palmitic acid was likewise used for the synthesis of complex lipids in wt and MDR cells and was incorporated mainly into PC (35.9 and 41.1%, respectively), PE (9.4 and 9.5%, respectively), and SM (3.4 and 6.9%, respectively). However, biosynthesis of lipid-1 and -2 from palmitic acid precursor was visible only in MDR cells (Fig. 3B). The autoradiograph in Fig. 3B also shows that MCF-7-wt cells incorporate radioactivity into a neutral lipid (lowermost spot, neutral lipid area). The comigration of this neutral lipid with oleoyl alcohol (solvent system IV) indicates that it is a fatty alcohol (data not shown). This is in accordance with previous work showing that fatty alcohol accumulates in MCF-7 wt cells but not in MDR MCF-7 variants (24). Data of a more qualitative nature were obtained from experiments using [3H]galactose. Fig. 3C shows that [3H]galactose was utilized by MDR cells for synthesis of lipid-1 and -2. Radioincorporation was markedly pronounced in MCF-7-AdrR cells; MCF-7-wt cells demonstrated slight incorporation into lipid-1 and no incorporation into lipid-2. Collectively, the data (Fig. 3) suggest that lipids-1 and -2, accumulating in MDR cells, are glycosphingolipids. A comparison of the migration of commercial glucosylceramides (Gaucher's spleen) with lipid-1 and -2 radioactivity revealed a comigration. Work in other cell types has amply demonstrated the central position played by glycosylceramide in glycosphingolipid synthesis (12, 13, 25). From the data of Fig. 3C it also appears that elevated glycosylceramide in MDR cells leads to enhanced formation of higher glycosphingolipids (lactosylceramide, gangliosides).

To verify that lipid-1 and -2 synthesis originates from a pathway involving sphingoid bases, sphinganine was added exogenously to the cell culture medium. As shown in Fig. 4, sphinganine supplementation caused a time-dependent elevation in the mass of lipid-1 in MCF-7-wt cells and elevation in the mass of lipid-1 and -2 in MCF-7-AdrR cells (detected by TLC charring). In addition, whereas lipid-1 from both cell lines migrated with an R_1 value of 0.53, lipid-2 did not appear in MCF-7-wt cells. Instead, synthesis of lipid X increased, indicating that lipid X is a sphingolipid.

Effect of Inhibitors of Sphingolipid Biosynthesis on Lipid-1 and Lipid-2 Accumulation—We used MCF-7-AdrR cells labeled

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**Fig. 2.** Thin-layer chromatographic char of lipids from MCF-7-wt and MCF-7-AdrR cells. Extracted lipids, 100 μg lane, were resolved by TLC using solvent system II. Lipids were visualized by H_2SO_4 charring as described under “Experimental Procedures” and identified by migration with commercial standards. X, unknown lipid.

**Fig. 3.** Autoradiograph of lipids from MCF-7 cells incubated with radiolabeled sphingolipid precursors. MCF-7-wt and MCF-7-AdrR cells, grown to near-confluency in 100 x 20-mm culture dishes, were labeled with [3H]serine ([3H]ser) (A), [3H]palmitic acid ([3H]pal) (B), or [3H]galactose ([3H]gal) (C), for 24 h in RPMI 1640 medium containing 0.1% BSA. Equal aliquots (based on uptake of radioactivity) of extracted cell lipids were analyzed by TLC using solvent system I (panels A and B) and solvent system III (panel C). The autoradiography of a representative chromatogram is shown.

**Fig. 4.** Metabolism of sphinganine by MCF-7-wt and MCF-7-AdrR cells. Cells, in 100 x 20-mm culture dishes, were incubated with 5.0 μM sphinganine/BSA (prepared at a 1:1 molar ratio) for the times indicated. Lipids were extracted, and a 100-μg lipid aliquot of each sample was resolved by TLC in solvent system II. The TLC plate was charred as described under “Experimental Procedures.” The data shown are representative of two independent experiments that gave similar results.
with [3H]palmitic acid or [3H]serine to investigate the effects of various inhibitors of sphingolipid biosynthesis. The first enzymatic reaction in sphingolipid base biosynthesis, catalyzed by 3-ketosphinganine synthase, is the formation of 3-ketosphinganine from serine and palmitoyl-CoA (Fig. 1A). Preincubation of cells with L-cycloserine, an inhibitor of 3-ketosphinganine synthase, caused almost complete disappearance of lipid-1 and major reduction in lipid-2 levels (Fig. 5A). Ceramide synthesis, which catalyzes formation of ceramide via an acylation reaction, can be inhibited by FB1 (11, 15, 26). Preincubation of MCF-7-AdrR cells with FB1 caused a profound reduction in the levels of lipid-1 and -2 (Fig. 5B). In further experiments, the glycolipid nature of the accumulating lipids in MDR cells was investigated using PPMP, an inhibitor of glucosylceramide synthase (12, 13, 15, 26). As shown in Fig. 5C, PPMP blocked completely the formation of lipid-1 and -2. This effect was accompanied by an elevation in ceramide levels and a reduction in lactosylceramide and ganglioside levels (Fig. 5C), reflecting the ability of PPMP to block glycolipid synthesis distal to glucosylceramide. These observations suggest that the metabolic steps governing the accumulation of lipid-1 and -2 are closely associated with glycosylation/deglycosylation events.

Identification of Glycosylceramide Structural Components—In order to definitively establish structure, TLC-purified preparations of lipid-1 and -2 were analyzed by FAB/MS. As shown in Fig. 6A, the upper band (lipid-1) had a somewhat heterogeneous FAB/MS spectrum. This band appeared to contain predominantly N-tetradecanoyl monoglucosylceramides in the cluster at (M + H)+/z 809/811 (precisely, N-tetradecanoyl (lignoceryl) monoglucosylceramide at (M + H)+/z 811 and N-tetradecanoyl (nervonoyl) monoglucosylceramide at (M + H)+/z 809) but also had peaks of (M + H)+/z 783 (N-docosanoyl) 723 (N-linoleoyl) monoglucosylceramides, (M + H)+/z 539 (N-palmitoyl ceramide), 567 (N-stearoyl ceramide), and 615 (N-docosatrienoyl ceramide). The heterogeneity and increased hydrophobicity of the lipid-1 peak, due to a larger inclusion of longer amide side chains, may account for its higher TLC migration. The lower TLC band (lipid-2) had a FAB/MS spectrum highly characteristic of N-palmitoyl monoglucosylceramide (Fig. 6B). The predominant peak of 699 was the native ion, with a well-defined N-palmitoyl ceramide breakdown peak of (M + H)+/z 537. This peak appeared to be relatively uniform, with a small amount of other expected monoglucosylceramides with different amide chains (e.g. (M + H)+/z 721 (N-linolenyl), 781 (N-docosanoyl), and 809/811 (N-tetradecanoyl)). These results confirm that MCF-7-AdrR cells have two major glycosylceramide species differing in their fatty acid constituents.

The carbohydrate headgroup identity of the TLC-purified glucosylceramides was examined by comparing the migration of the lipids with that of commercial lactosyl-, galactosyl-, and glucosylceramide standards. As shown in Fig. 7, glycosphingolipid standards were separated according to their carbohydrate moiety (lanes 1-3). The migration of the cell-purified glycosylceramide doublet (lane 4) was aligned with that of glucosylceramide. These results identify the accumulated glucosylceramides as glucosyl- rather than galactosylceramides.

Time Course for Formation of Glucosylceramides—[3H]Palmitic acid was used to determine the time course of lipid formation. As shown in Fig. 8A, uptake and incorporation of [3H]palmitic acid was nearly equal in MCF-7-wt and MCF-7-AdrR cells. Fig. 8B shows that [3H]palmitic acid was rapidly incorporated into ceramide, with 3.6-fold higher levels in MCF-7-AdrR cells than in MCF-7-wt cells at 30 min. Thereafter (1-6 h), the levels of [3H]ceramide decreased similarly in both cell lines, reflecting conversion of ceramide to sphingolipids. The incorporation of [3H]palmitic acid into glucosylceramides showed a strikingly different pattern. This diversity was characterized by a consistently higher rate of glucosylceramide formation in MCF-7-AdrR cells, which reached a maximum of 3.1% of total lipid tritium at 6 h (Fig. 8C). In contrast, glucosylceramide formation in MCF-7-wt cells was significantly lower, accounting for only 0.38% of total lipid tritium at 6 h. These data show an 8-fold difference in the rate of glucosylceramide formation in wt and MDR cell types. The level of other specific lipids in the two cell lines was also compared. As shown in Table I, MCF-7-wt cells showed slightly higher incorporation of [3H]palmitic acid into PC at 1 h (1.7-fold), a difference that was equalized by 6 h. Similar results were obtained for PE. While...
glycerophospholipid metabolism was alike in both cell lines, SM formation was higher in MCF-7-AdR cells (3.4- and 1.9-fold at 1 and 6 h, respectively) (Table I). These results are in agreement with previous work (27). Because sphingomyelin synthase utilizes ceramide as a substrate for SM synthesis, the higher levels of radiolabeled SM in MCF-7-AdR cells may be due to enhanced ceramide formation (Fig. 8B). Collectively, the data of Table I show that the major difference in lipids between MCF-7-wt and MCF-7-AdR cells is in glucosylceramide levels.

Glucosylceramide Degradation Rates—The accumulation of glucosylceramides in MCF-7-AdR cells is a consequence of either increased synthesis or decreased degradation. To test the possibility that glucosylceramide degradation is altered in the MDR cells, MCF-7-wt and MCF-7-AdR cells were labeled with [3H]galactose for 24 h and then chased in [3H]galactose-free medium for an additional 6, 12, and 24 h. As shown in Fig. 9, radiolabeled glucosylceramide levels were decreased in both cell lines in a similar fashion. In light of the high amounts of glucosylceramide in MCF-7-AdR cells, it is notable that degradation rates were slightly accelerated in this cell line. These findings indicate that the accumulation of glucosylceramides in MCF-7-AdR cells is due to increased synthesis and is not a consequence of hindered breakdown.

Accumulation of Glucosylceramides in Other MDR Cells—In addition to MCF-7-AdR cells, glucosylceramide accumulation was observed in other MDR cell lines. Fig. 10 shows the TLC lipid profile of OVCAR-3 (MDR ovarian carcinoma), KB-3–1 (drug-sensitive, wt), and KB-V-1 (MDR) epidermoid carcinoma cells. The MDR cell lines OVCAR-3 and KB-V-1 most strikingly demonstrate the glucosylceramide doublet. The ovarian carcinoma is resistant to clinically relevant concentrations of Adriamycin, melphalan, and cisplatin (28). These data imply that the association of elevated glucosylceramide levels with multidrug resistance is more global as opposed to a biochemical characteristic of limited scope. As such the work poses interesting questions regarding a biological role for glycosphingolipids in the ability of cells to resist drug toxicity.

**DISCUSSION**

We have shown, for the first time, the accumulation of glucosylceramides in cells expressing the multidrug resistance phenotype. These compounds were readily radiolabeled by pre-incubation of cells with glycosphingolipid precursors, and their biosynthesis was sensitive to sphingolipid biosynthesis inhibitors. The lipids contained a long-chain sphingoid base, fatty acids of either 16 (palmitic) or 24 (nervonic, lignoceric) carbons, and glucose. Palmitic, nervonic, and lignoceric acids are among the most common aliphatic species comprising sphingolipids (29).

Earlier studies have endeavored to distinguish drug-sensitive and drug-resistant cells by differences in sphingolipid synthesis and composition. Reported differences in lipid composition of doxorubicin-sensitive and -resistant P388 cells were mainly confined to triglycerides with minor changes in SM and PC in drug-resistant cells (27). Our data show similar minor alterations with respect to phospholipid and SM levels (Table I). Biedler and co-workers (30) examined ganglioside composition in daunorubicin-resistant, vincristine-resistant, and drug-sensitive cells. Although differences in ganglioside composition were found, there was no definitive correlation with drug resistance. Another study examined the levels of four major lipid classes, including gangliosides, in daunorubicin-sensitive and -resistant P388 cells (31). No differences in lipid composition were noted (31). Our study provides the first evidence for...
Multidrug resistance-associated alteration in glucosylceramide

MDR and wt cells are in order. Assays addressing the relative activity of this enzyme in MCF-7-AdrR cells is glucosylceramide synthase. Another enzyme that may be involved in accelerated glycosphingolipid synthetic pathway exists in the MDR MCF-7-wt cells. These results indicate that a more active glucosylceramide synthase (14–16, 35), transformation (17, 18), and tumor metastasis (18, 36, 37). Changes in expression of various glycosphingolipids on the cell surface have been correlated with mechanisms of acquiring and maintaining cancer phenotype and tumor progression (17, 18). For example, human melanoma expresses GD2 ganglioside, and the level of GD2 increases as melanoma tumorigenesis progresses (38). This increase may be correlated with metastatic potential, as GD2 has been implicated in the attachment of melanoma cells to solid substrata (37). A novel sialylated fucosyl glycosphingolipid has been characterized in chronic myelogenous leukemia cells (38), and occurrence of another ganglioside, GD1α, has been associated with rat ascites hepatoma AH 7974F cells (39).

The multiple putative functions of glycosphingolipids in cell growth and transformation suggest that glucosylceramides, in light of our findings with MCF-7-AdrR cells, exert a role in acquiring and/or maintaining multidrug resistance. Elevated glucosylceramide levels were likewise expressed in other MDR cells we have studied (Fig. 10). Therefore, the biochemical processes underlying this accumulation are not restricted to breast cancer and/or to Adriamycin resistance. The contribution of glycosphingolipids may be important for MDR cells to survive a hostile environment (33, 40, 41). Interestingly, drugs that inhibit glycosphingolipid synthesis may elicit cytotoxic activity. For example, recent work has shown that 3'-azidothymidine alters glycosphingolipid metabolism in K562 erythro-leukemia cells, an effect that may be related to cytotoxicity (41). The accumulation of glucosylceramides in MDR cells may impart resistance to toxic insult and thereby enhance cell survival. As such, P-gp activity, suggested to be dependent upon lipid environment (42–44), may be regulated by glucosylceramides.

| Lipid     | 1 h     | Incubation time | 6 h     |
|-----------|---------|-----------------|---------|
|           | MCF-7-wt | MCF-7-AdrR      | MCF-7-wt | MCF-7-AdrR |
| PC        | 28.3 ± 0.45 | 16.1 ± 0.73    | 20.6 ± 2.83 | 19.8 ± 2.70 |
| PE        | 4.45 ± 0.14  | 3.02 ± 0.05    | 3.50 ± 0.06 | 3.72 ± 0.01 |
| SM        | 0.55 ± 0.06  | 1.91 ± 0.10    | 1.34 ± 0.32 | 2.50 ± 0.23 |
| Glu-cer   | 0.18 ± 0.02  | 1.50 ± 0.07    | 0.38 ± 0.03 | 3.10 ± 0.19 |

Fig. 10. Qualitative analysis of lipids from wild-type and multidrug-resistant cancer cell lines. Cells were grown to near-confluency in their respective media. Lipids were extracted from the cells as indicated under "Experimental Procedures." Equal amounts of total lipid (50 μg/lane) were separated by TLC using solvent system I. The lipids were visualized and identified as described in the legend to Fig. 2. Abbreviations: SM, sphingomyelin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; OV, NIH:OVCAR-3 cells (MDR); 3-1, KB-3-1 cells; V-1, KB-V-1 cells (MDR).

Table I

Incorporation of [3H]palmitic acid into PC, PE, SM, and glucosylceramide of MCF-7-wt and MCF-7-AdrR cells

Cell cultures were incubated with [3H]palmitic acid for 1- and 6-h periods in medium containing 5% FBS, and total lipids were extracted and analyzed. PC, PE, and SM were separated by TLC using solvent system IV. Glucosylceramides (Glu-cer) (lipid-1 and -2) were separated by TLC using solvent system I. After TLC resolution, quantitation of radiolabel in the relevant regions of the TLC plate was conducted as detailed under "Experimental Procedures." Data represent the amount of each lipid as a percentage of the total radiolabeled lipid tritium. The remaining radioactivity was localized in neutral lipids and other phospholipids. Data are from one of three experiments that gave similar results.

PC 28.3 ± 0.45 16.1 ± 0.73 20.6 ± 2.83 19.8 ± 2.70
PE 4.45 ± 0.14 3.02 ± 0.05 3.50 ± 0.06 3.72 ± 0.01
SM 0.55 ± 0.06 1.91 ± 0.10 1.34 ± 0.32 2.50 ± 0.23
Glu-cer 0.18 ± 0.02 1.50 ± 0.07 0.38 ± 0.03 3.10 ± 0.19

Glucosylceramide Accumulation in MDR Cells

It is clear that glucosylceramide accumulation in MDR cells may be due to increased activity of the enzyme that synthesizes glucosylceramide. This is supported by the finding that the activity of glucosylceramide synthase is higher in MCF-7-AdrR cells than in MCF-7-wt cells. The increased synthesis of glucosylceramide in MCF-7-AdrR cells is consistent with the observation that multidrug resistance-associated alteration in glucosylceramide levels.

As judged by the time course of [3H]palmitic acid labeling (Fig. 8), the rate of glucosylceramide synthesis is much higher in MCF-7-AdrR cells than in MCF-7-wt cells. Such differences can be explained by defects in glycosphingolipid degradation pathways, as in fibroblasts from patients with Gaucher’s disease (32, 33). However, pulse-chase experiments (Fig. 9) ruled out this possibility, and demonstrated that glucosylceramide degradation rates in MCF-7-AdrR cells were even higher than in MCF-7-wt cells. These results indicate that a more active glucosylceramide synthase exists in MDR MCF-7-AdrR cells. Other enzymes that may be involved in accelerated glycosphingolipid synthesis in MCF-7-AdrR cells is ceramide synthase. Recent work by Bose et al. (34) shows that daunorubicin stimulates ceramide elevation in P388 and U937 cells via activation of ceramide synthase. Another enzyme that may contribute to the enhanced synthesis of glucosylceramide in MCF-7-AdrR cells is glucosylceramide synthase. In vitro enzymatic assays addressing the relative activity of this enzyme in MDR and wt cells are in order.

Glucosylceramides play a role in cell growth (14, 15, 25), differentiation (14–16, 35), transformation (17, 18), and tumor metastasis (18, 36, 37). Changes in expression of various glycosphingolipids on the cell surface have been correlated with mechanisms of acquiring and maintaining cancer phenotype and tumor progression (17, 18). For example, human melanoma expresses GD2 ganglioside, and the level of GD2 increases as melanoma tumorigenesis progresses (38). This increase may be correlated with metastatic potential, as GD2 has been implicated in the attachment of melanoma cells to solid substrata (37). A novel sialylated fucosyl glycosphingolipid has been characterized in chronic myelogenous leukemia cells (38), and occurrence of another ganglioside, GD1α, has been associated with rat ascites hepatoma AH 7974F cells (39).

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Glucosylceramide Accumulation in MDR Cells

We propose that glucosylceramides may be used as an index to identify MDR cancer.

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