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Inhibition of glycogen phosphorylase (GP) by CP-91,149 induces growth inhibition correlating with brain GP expression☆

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Abstract

The role of glycogenolysis in normal and cancer cells was investigated by inhibiting glycogen phosphorylase (GP) with the synthetic inhibitor CP-91,149. A549 non-small cell lung carcinoma (NSCLC) cells express solely the brain isozyme of GP, which was inhibited by CP-91,149 with an IC_{50} of 0.5 μM. When treated with CP-91,149, A549 cells accumulated glycogen with associated growth retardation. Treated normal skin fibroblasts also accumulated glycogen with G1-cell cycle arrest that was associated with inhibition of cyclin E-CDK2 activity. Overall, cells expressing high levels of brain GP were growth inhibited by CP-91,149 correlating with glycogen accumulation whereas cells expressing low levels of brain GP were not affected by the drug. Analyses of 59 tumor cell lines represented in the NCI drug screen identified that every cell line expressed brain GP but the profile was dominated by a few highly GP expressing cell lines with lower than mean GP-a enzymatic activities. The correlation program, COMPARE, identified that the brain GP protein measured in the NCI cell lines corresponded with brain GP mRNA expression, ADP-ribosyltransferase 3, and colony stimulating factor 2 receptor α in the 10,000 gene microarray database with similar correlation coefficients. These results suggest that brain GP is present in proliferating cells and that high protein levels correspond with the ability of CP-91,149 to inhibit cell growth.

Keywords: Brain glycogen phosphorylase; CP-91,149; Tumor; Cancer; Cell cycle; Glioma; Colony stimulating factor α

The role of glycogen metabolism in cell proliferation and tumor formation is not well studied, even though altered regulation of carbohydrate metabolism has long been associated with cancer cells [1,2]. During tumor progression, glycogen content was found to vary depending on advancement of the disease, for example, preneoplastic liver cancer showed a higher glycogen content than the neoplastic stage suggesting a function for glycogen in early development of liver cancer [3]. Regulatory enzymes catalyzing glycogen degradation and synthesis are glycogen phosphorylase (GP) and glycogen synthase (GS), respectively. Both of these key metabolic enzymes are regulated by covalent phosphorylation and by allostery [4,5]. There are three isoforms of GP; brain, liver, and muscle GP, which are expressed in the specific tissues [6,7]. The brain GP isozyme is the predominant fetal isozyme in normal rodent and human tissues except in liver [8,9]. Moreover, brain GP is expressed in early stages of gastric and colorectal cancers, but not in the normal tissues [10]. By contrast, breast cancer cells overexpressing Her2/Neu had reduced protein levels of brain GP [11].

Previously, we identified that the synthetic flavone, flavopiridol, binds to different GP isoforms and inhibits brain GP in tissue culture and the purified rabbit muscle GP [12,13]. Crystallization of flavopiridol with muscle GP determined that the drug binds to the enzyme's

☆ *Abbreviations:* GP, glycogen phosphorylase; CDK, cyclin-dependent kinase; NSCLC, non-small cell lung carcinoma.

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purine nucleoside site and inhibits GP by an allosteric mechanism similar to that of caffeine [14]. Several other cytotoxic drugs including caffeine, curcumin, K252a or staurosporine also inhibit either GP or the glycogen phosphorylase kinase (PHK), which activates GP [15,16]. Abnormal activation of PHK is associated with psoriasis, a hyperproliferative skin disease. Inhibition of PHK has led to treatment of this disorder indicating that activated GP may participate in growth regulation of normal epidermoid cells [17]. Recently, an inhibitor of the human liver GP isozyme, CP-91,149, was designed for diabetic therapy [18]. CP-91,149 stabilizes the inactive conformation of human liver GP by binding at a site located at the subunit interface in the region of the central cavity of the dimeric structure [19,20].

In this paper, analyses of 59 NCI cell lines demonstrate that the brain GP isoform is the predominant isozyme expressed in cancer lines and that CP-91,149 inhibits brain GP. Growth inhibition by CP-91,149 correlates with high expression of brain GP and increase in glycogen content.

Materials and methods

Cell lines and cell culture. HSF55 normal skin fibroblasts (Biosciences Los Alamos, passage 18), SV40-transformed skin fibroblasts CT-10-2C-T1 (Biosciences Los Alamos), normal rat astrocytes isolated from rat cerebellum (Kim McAllister, Department of Neurology, UC Davis), the rat C6 glioma, the human gliomas T98G (ATCC) and U87 (ATCC), and the A549 NSCLC (ATCC) were used in this study. Pellets from the 59 cell lines from the drug screen were provided by the Developmental Therapeutics Program of NCI. All human cells were grown in regular RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum and with penicillin (100 U/ml) and streptomycin (100 μg/ml), while rat cells were grown in DMEM.

Flow cytometric analysis. Cells were fixed with 70% ethanol. DNA was stained with propidium iodide and the intensity of fluorescence was measured using a Becton–Dickinson flow cytometer at 488 nm for excitation and at 650 nm for emission [21]. The cell cycle profile was analyzed using Modifit's Sync Wizard (Verity Software).

Immunoblot analysis. Forty microgram of cell extract was separated on a 12% SDS–polyacrylamide gel, transferred to a nylon membrane, and probed with polyclonal antisera raised against CDK2, CDK4, cyclins A, D1, E, p21cip1 (Santa Cruz Biotechnology) human brain [22], muscle [23], and liver isozymes of GP, or monoclonal antibodies raised against p27kip1 (Pharmingen). The antibodies against human liver GP were raised in rabbits using the peptide NH_2–CASKTG STRGAGTVF–COOH (Genmed). Detection was performed by using secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) coupled to the ECL detection system (NEN Life Science Products). Expression of brain GP in the 59 cell lines was quantified by normalizing the band intensities for brain GP to actin on the autoradiographs, which had been exposed for the same amount of time.

GP enzyme assays. For determination of GP enzyme activity cell pellets from the 59 cell lines were lysed in 100–200 μl lysis buffer containing 10 nM calyculin A [24]. The protein concentration was determined with the Bradford assay (Biorad). Cell extract (50 μl) was used to measure GP activity according to Layzer et al. [25]. Kinetics were followed for 5 min with 30 s intervals. The GP activity is expressed as U/min/mg protein.

For the determination of the IC_{50} value of CP-91,149 for inhibition of brain GP about 0.5 ml packed A549 cells were used and lysed to give a final concentration of 10 μg protein/μl. 125 μg protein was used for each point. CP-91,149 was diluted to stock solutions and 1 μl of the corresponding stock solutions was added to the assay mix and preincubated for 2 min at room temperature prior to starting the reaction with glycogen. All measurements were done in triplicate.

CDK2 kinase assays. Cyclin E-CDK2 activity was measured as previously described [24]. Briefly, 2 μg of anti-cyclin E antibodies was used to immunoprecipitate cyclin E from 200 μg cell extract followed by a H1 kinase assay. The cold ATP concentration was 10 μM and the assay time was 15 min at 30 °C.

Determination of the glycogen content. Cells were seeded at approximately 0.75×10^6 cells/100 mm dish and allowed to attach overnight before treatment with CP-91,149. Cells were harvested by trypsinization and further processed as described [9]. The glycogen content was measured with some modifications using anthrone (Sigma) [26]. Briefly, trypsinized cells from one dish were centrifuged, resuspended in 0.4 ml of 30% KOH, and incubated for 15 min at 95 °C to extract glycogen. Then 0.2 ml of 2% $NaSO_4$ was added and the glycogen was precipitated with 1.2 ml ethanol. The precipitate was resuspended in 1 ml H_2O:sulfuric acid (3:7.6 ratio) containing 0.15% anthrone and heated for 15 min at 95 °C and the reaction was stopped by chilling the tubes in ice. The glycogen content was immediately determined spectrophotometrically at 620 nm.

COMPARE methodology. The publicly available data concerning the chemosensitivity and molecular target profiles of the cell lines from the NCI anticancer drug screen can be accessed at the URL http://dtp.nci.nih.gov/. The NCI screening procedures have been described previously [27,28]. For this investigation, the pattern of GP activity and protein expression in 59 human tumor derived cell lines were graphically represented through the mean graph [29] by plotting individual measurements relative to the average of all measurements: Deflections to the left of central mean are those cell lines with less than average values of the GP while those deflected to the right are cell lines with more than average expression/activity. The pattern of expression of these molecular targets was correlated with the sensitivity profiles of test compounds and available molecular target profiles using the COMPARE algorithm [29]. COMPARE analyzes the different compound (standard anticancer agents, synthetic compounds, natural compounds, and molecular targets) and target databases to search for the highest ranked compounds or molecular targets matching the distribution of the input data across the panel of cell lines. The COMPARE analyses are reported as rank-ordered lists of compounds/targets characterized by the Pearson correlation coefficient, which measures the yield of similarity between the seed and correlate.

Results

CP-91,149 inhibits brain GP and causes glycogen accumulation in A549 cells

CP-91,149 had originally been designed as an inhibitor for human liver GP, but all three GP isoforms demonstrate high primary protein sequence homologies at the intersubunit site shown to complex with CP-91,149 [19]. The brain isozyme of GP is expressed in several transformed cell lines while muscle GP has not been reported to be expressed in proliferating cells [6,9]. Recently, we demonstrated that the A549 NSCLC cell line expresses brain GP [9] while the liver and muscle isozymes are expressed negligibly or at levels below

detection (Fig. 1A). To examine if brain GP is inhibited by CP-91,149, cell extracts were prepared from A549 cells and increasing amounts of the inhibitor were added to the cell extract in the absence of allosteric effectors (Fig. 1B). We determined 50% inhibition of enzymatic activity at 0.5 μM CP-91,149 demonstrating inhibition of brain GP. Inhibition of brain GP by CP-91,149 was further examined in tissue culture by treating A549 cells with different concentrations of CP-91,149 for 72 h. A significant increase in glycogen accumulation was detected at 10 μM of CP-91,149 as compared with untreated cells with a maximal glycogen accumulation at 30 μM (Fig. 1C). Intracellular glycogen content decreased at 50 μM CP-91,149, perhaps explained by additional pharmacological effects of the drug. The dose-dependent accumulation of intracellular glycogen in A549 cells by CP-91,149 indicates that CP-91,149 inhibits brain GP in tissue culture. In the enzyme assay, 30 μM CP-91,149 inhibited GP activity completely and corresponded closely with glycogen accumulation in tissue culture cells.

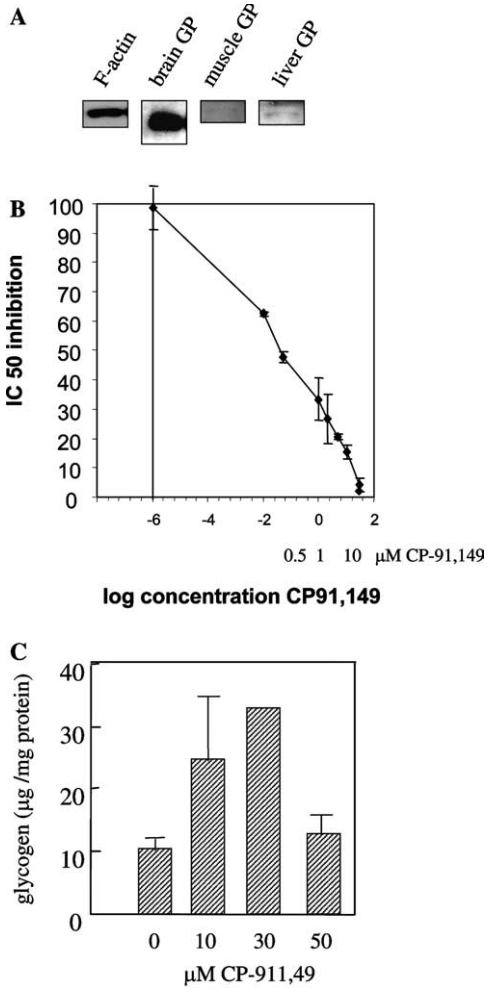

Fig. 1. Glycogen and GP analysis in A549 NSCLC cells in response to CP-91,149. (A) Immunoblot analysis of the different GP isoforms in A549 cells is shown. 40 μg total cell extract was separated by SDS–PAGE. (B) In vitro GP assay with cell extract from A549 cells. Total cell extract from one batch of A549 cells was prepared and the assays were performed as described in Materials and methods. (C) Determination of glycogen levels. A549 cells were left untreated (0) or treated with 10, 30, and 50 μM CP-91,149 for 72 h.

Differential effect of CP-91,149 on growth of normal skin fibroblasts HSF55 and transformed fibroblasts CT-10-2C-T1

Normal human skin fibroblasts HSF55 and CT10-2C-T1 cells transformed with the SV40 large T-antigen were treated with different concentrations of CP-91,149 for 72 h. We observed an increase in glycogen content in HSF55 cells with a maximal effect at 30 μM of CP-91,149, as was observed with A549 (Fig. 2A). There was a coordinate inhibition of growth of HSF55 cells, which correlated with glycogen increase (Fig. 2B), despite a small difference between treated and untreated cell numbers, presumably due to the slow growth rate of HSF55 and their tendency to undergo density-induced growth arrest. By contrast, the basal glycogen content of SV40-transformed skin fibroblasts, CT10-2C-T1, was lower than in HSF55 cells. Treated CT10-2C-T1 cells demonstrated a small increase in intracellular glycogen content and were not growth inhibited. At 30 μM CP-91,149 an increase in the number of cells was observed (Fig. 2B).

Growth arrest of HSF55 was confirmed by FACS analysis where CP-91,149 treatment increased G1-phase cells with a significant reduction of the S-phase population (Fig. 2C). In contrast, treated CT10-2C-T1 cells did not demonstrate cell cycle arrest although some growth inhibition was observed at high doses (50 μM) of CP-91,149 that was not associated with glycogen accumulation. This effect was possibly the same unidentified effect of the drug as was observed with A549 cells. Both HSF55 and CT10-2C-T1 expressed brain and liver GP, with the protein level of brain GP higher in HSF55 cells (Fig. 2D).

Cell cycle protein analysis of HSF55 cells treated with CP-91,149

The expression of cell cycle regulatory proteins was analyzed by immunoblot to better elucidate mechanisms of the cell cycle arrest observed in HSF55 cells treated with CP-91,149 (Fig. 3A). There was unaltered expression of cyclins D1 and E and reduced expression of cyclin A when cells were treated with CP-91,149. The reduction in cyclin A demonstrates G1-phase arrest, since cyclin A is solely expressed in S- and G2- and at the beginning of M-phase [30]. The cyclin-dependent kinase inhibitors p21cip1 and p27kip1 were increased in treated HSF55 cells, as compared with untreated control cells. The increased expression of p21 and p27

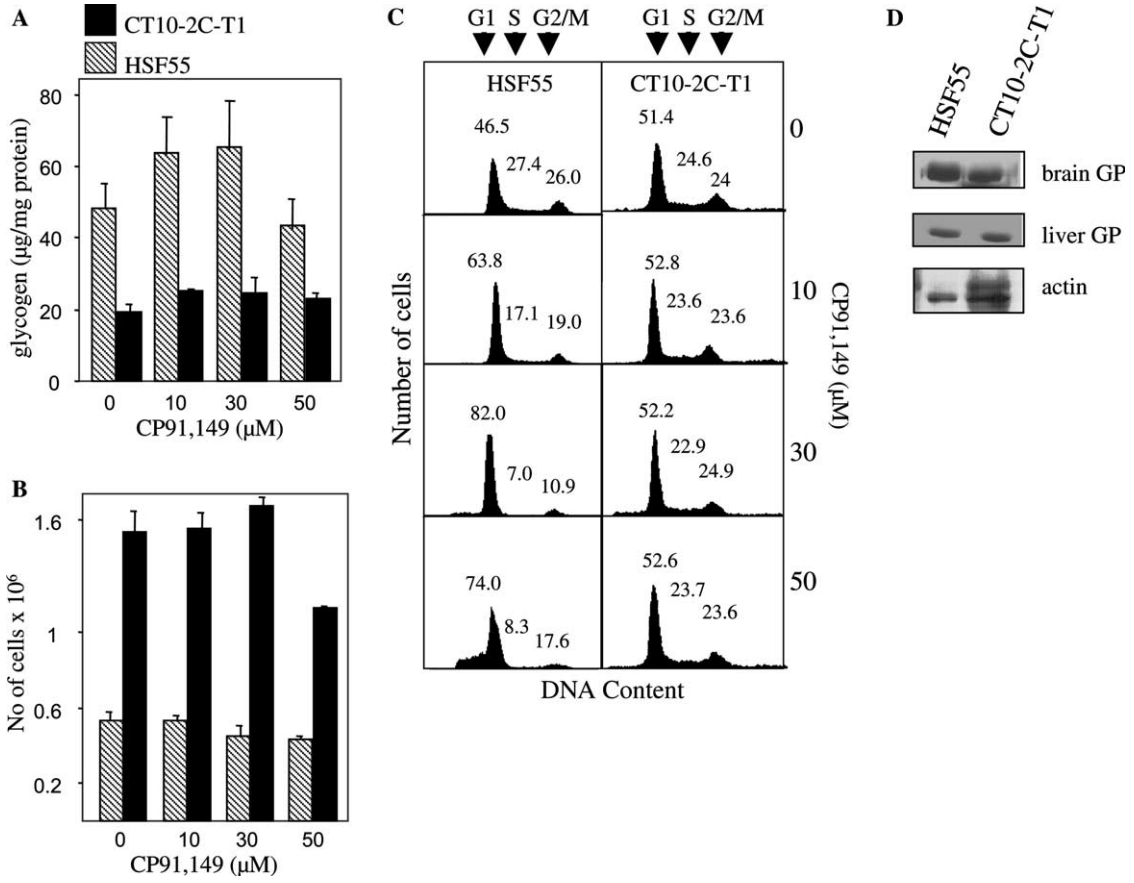

Fig. 2. Analysis of normal fibroblasts HSF55 and transformed fibroblasts CT10-2C-T1 cells treated with CP-91,149. (A) The glycogen content was determined in untreated and in cells treated with CP-91,149 for 72 h. (B) Viability was determined with manual cell counts following staining with trypan blue exclusion assay. The number of viable cells was determined. (C) The DNA content of HSF55 was quantitated using FACS analysis. The cell sorter populations were analyzed using χ^2. There was a inverse relationship between the CP-91,149 concentration and cell cycle distribution ($\chi^2 = 3469$, df = 6, $p < 0.001$). (D) Immunoblot analysis of different GP protein isoforms expressed in HSF55 and CT10-2C-T1.

corresponds with the observed decreased cyclin E-CDK2 kinase activity (Fig. 3B) suggesting a typical cell cycle arrest in late G1-phase.

Analysis of normal and tumor cells following GP inhibition by CP-91,149

The possible association between glycogen accumulation and growth inhibition was investigated involving treatment of normal rat primary astrocytes, three transformed glioma cell lines, human T98G, U87, the rat C6 glioma, and the A549 NSCLC cell line, with 30 µM CP-91,149. Treated astrocytes, T98G and A549, accumulated glycogen and demonstrated growth inhibition in the presence of CP-91,149. The extent of glycogen accumulation and growth inhibition differed among the cell lines (Figs. 4A and B). The highly malignant U87 and C6 glioma cell lines did not accumulate glycogen nor show any growth reduction or arrest in the presence of CP-91,149. Growth inhibition of cells by CP-91,149 was always associated with glycogen accumulation, which was not observed in drug-resistant cells, thus providing evidence for a relationship between inhibition of glycogen degradation and growth inhibition. Immunoblot measurement of GP expression demonstrated that all cell lines tested expressed brain GP, but not the liver and muscle isozymes (Fig. 4C). However, there was a correspondence observed between levels of the brain GP protein and growth inhibition by CP-91,149. Astrocytes and T98G expressed the highest level of brain GP followed by A549 cells, whereas U87 and C6 glioma expressed low levels of brain GP.

Brain GP protein expression in 59 cell lines from the NCI drug screen

Our initial data of the cell line analysis suggested that brain GP may be the principal GP isoform in proliferating cells. However, only a few cell lines have so far been analyzed. HSF55 and CT10-2C-T1cells both express brain and liver isozymes of GP, but protein levels of brain GP were greater in HSF55 cells (Fig. 2D). Furthermore, astrocytes and T98G expressed the highest level of brain GP followed by A549 cells (Fig 4C). These

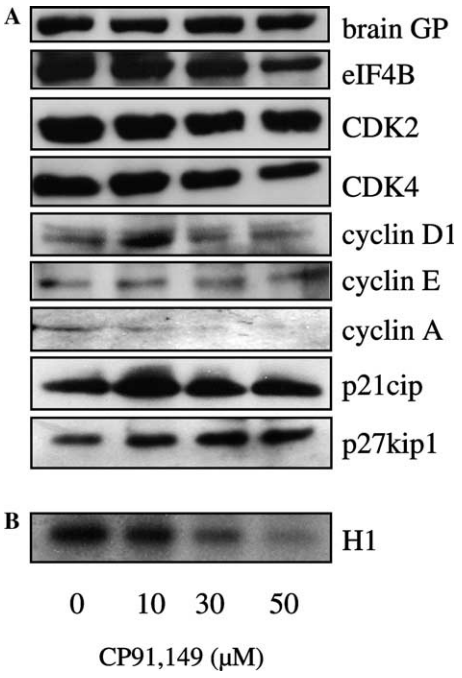

Fig. 3. Cell cycle protein analysis of HSF55 cells treated with CP-91,149. (A) Different cell cycle proteins in cells from untreated and CP-91,149 treated cells were analyzed by immunoblot. Forty microgram total protein was size-fractionated by SDS–PAGE. (B) The cyclin E-CDK2 kinase activity was determined by immunoprecipitation of cyclin E. Histone H1 was used as a substrate. The radiolabeled H1 protein band was identified by autoradiography.

data suggested a potential relationship between brain GP protein levels, glycogen accumulation, and growth inhibition produced by CP-91,149. A search for other cellular proteins, whose expression correlates with up or downregulation of brain GP, could lead to the identification of important oncogenes, as has been shown experimentally for the Her2/Neu oncogene in breast cancer cells [11].

For these reasons we analyzed 59 cell lines from the NCI drug screen for expression of GPs. These human cell lines are derived from nine major histological types (bone marrow, lung, skin, ovary, colon, central nervous system, kidney, prostate, and breast) and are routinely used to evaluate preliminary activity of new compounds from synthetic and natural origins [27]. The comparison uses an algorithm, COMPARE [31], that determines similarity of patterns between that of the given 'seed' and others within the databases, by creating similarity indices that are quantitatively expressed as Pearson correlation coefficients (PCC). The output of the COMPARE program is a rank order list of the most highly correlated patterns (plus PCC) from the requested database. These include drug response databases, a molecular target database of protein or gene expression targets measured in the cell lines, and a searchable database of microarray data [32].

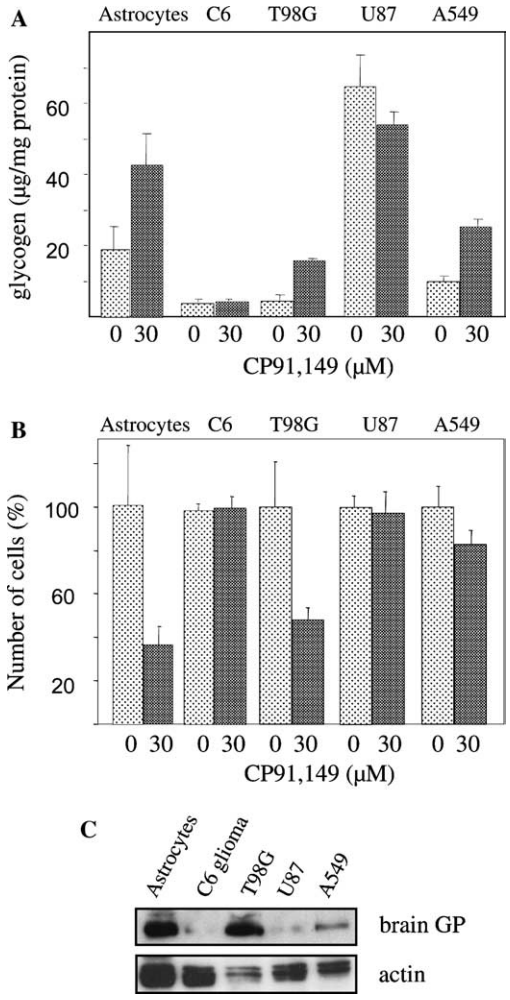

Fig. 4. Glycogen content and growth analysis of normal rat astrocytes, rat C6 glioma, and human T98G, U87, and A549 cells treated with CP-91,149. (A) The glycogen content was determined from cells incubated in the absence and presence of 30 μM CP-91,149 for 72 h. (B) The number of viable cells was determined by manual cell counts of cells briefly incubated with trypan blue. (C) Immunoblot analysis of brain GP from 40 μg of total cell extract from several cell lines.

Expression of brain, liver, and muscle GP was measured in the 59 cell lines by immunoblot. All cell lines expressed brain GP, a very few weakly expressed liver GP, but none expressed muscle GP. This suggests that brain GP is the isoform expressed in proliferating cells (Fig. 5A). High levels of brain GP expression were observed in two cell line clusters, colon (3 of 7) and melanoma (3 of 8). A549 and PC3 (unpublished data), previously shown to respond to CP-91,149 treatment, expressed brain GP at levels higher than the average expression for all 59 cell lines. Attempts were made to correlate GP expression data to patterns in the microarray database, which contains gene expression values for approximately 10,000 genes or transcripts in the 59 cell lines, based on comparative hybridization of RNA and a pooled RNA sample from 12 of the cell lines [32] to cDNA transcripts on a

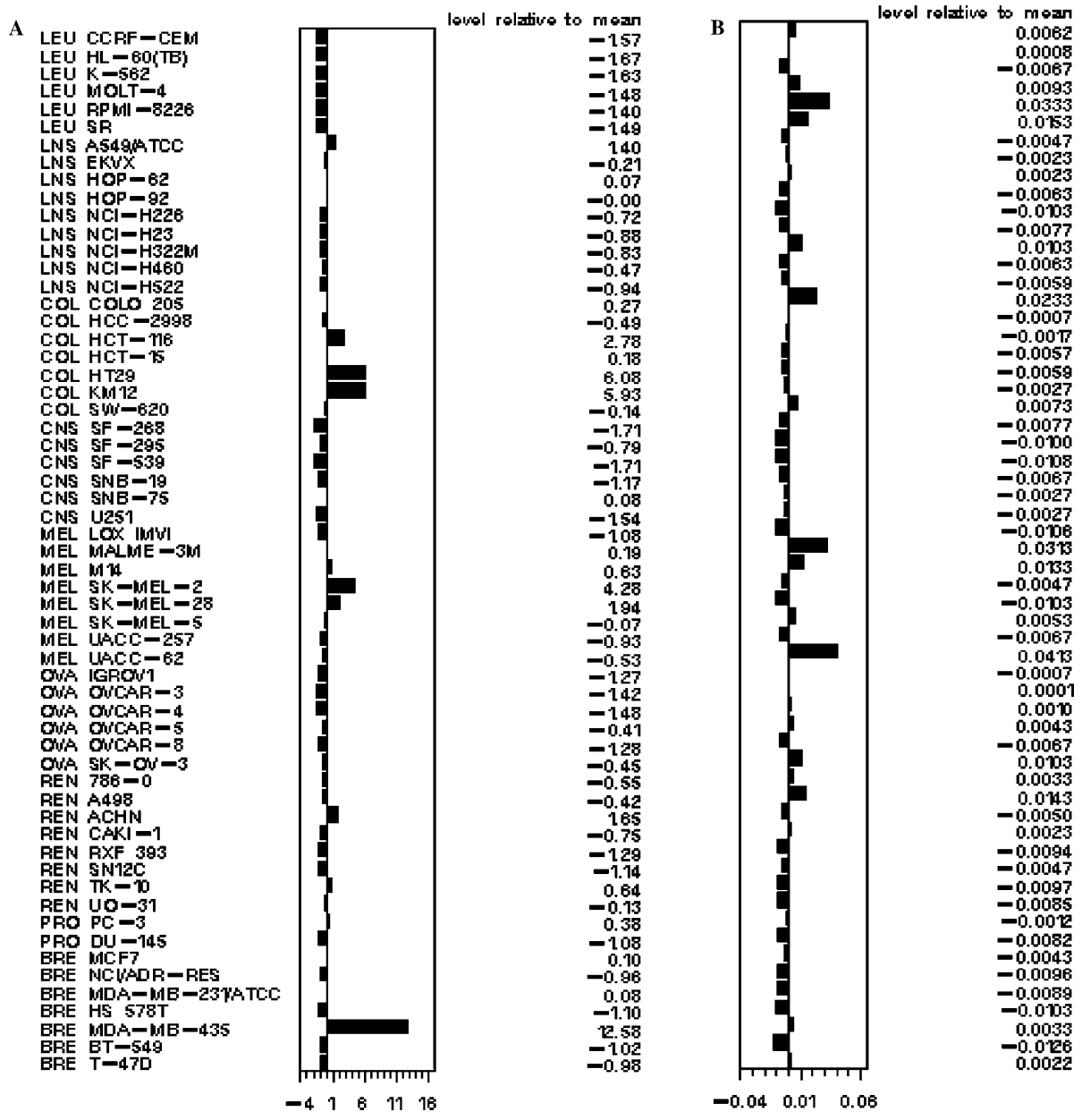

Fig. 5. GP analysis in 59 cell lines from the NCI drug screen. (A) Expression of brain GP protein levels normalized to F-actin in the 59 cell lines. Total cell extract was prepared from frozen samples of the 59 cell lines and 40 μg total cell extract was separated by SDS–PAGE. The band intensities for brain GP were quantitated from immunoblots normalized to the intensities of the F-actin bands. (B) GP activities in the 59 cell lines. Cell extracts from frozen cells of the 59 cell lines were prepared, and the GP activities were determined in the absence of allosteric activators for 5 min with 30 s intervals. The linear range of product formation was used to determine rates of product formation per minute per mg protein.

microarray chip. Statistical analysis was performed using SAS software package (SAS Institute, Carey, NC), and a statistically significant correlation was observed between the seed pattern of brain GP expression and the level of gene expression of a transcript identified as the brain form of GP from the microarray database, with a PCC of 0.53. The PCC value was transformed to standard normal z statistics and the associated one-sided p was value determined from published statistical tables and subjected to a Bonferroni correction ($p = 0.033$). A similar significant correlation was also observed with transcripts for ADP-ribosyltransferase and colony stimulating factor 2 receptor α. No correlation, however, was observed between the brain GP expression and the available agent drug responses. This result shows that the brain GP protein expression correlates well with the brain GP mRNA expression suggesting transcriptional regulation of brain GP.

Brain GP Activity in the 59 cell lines from the NCI drug screen

GP is activated by covalent phosphorylation (GP-a) with both forms, GP-a and GP-b, existing intracellularly. The GP activity in the absence of any allosteric activator (GP-a) was measured in the NCI cell line panel to determine if brain GP protein levels were related to intrinsic GP activities. From the mean graph data in Fig. 5B, clusters of higher than mean GP activity were observed in leukemia (5 of 6), melanoma (4 of 8), ovarian (4 of 6), and renal cancers (3 of 8). However, the majority of cell lines with increased GP-a activities demonstrated reduced levels of brain GP while most cell lines expressing increased brain GP demonstrated reduced GP-a activity indicating a possible inverse correlation that high brain GP protein level corresponds with low GP-a and high GP-b. Moreover, GP activity did not significantly correlate with any particular drug or drug target.

Discussion

The GP inhibitor CP-91,149 was used to investigate the contribution of glycogenolysis to cell growth of normal and transformed cells. CP-91,149 had been designed as an inhibitor for human liver GP, and this is the first report to our knowledge demonstrating its broader utility as an inhibitor of the brain GP isozyme. The inhibition of brain GP by CP-91,149 using cell extracts and the dose-dependent accumulation of glycogen in A549 cells treated with CP-91,149 are consistent with inhibition of brain GP in tissue culture cells. Alternatively, indirect enzymatic targets, such as phosphorylase kinase, could potentially be inhibited by the drug. However a larger number of potential targets including several diabetic targets were tested for inhibition by CP-91,149 and the test results had been negative [18]. Another explanation for the glycogen accumulation in treated cells is that cell cycle arrest could be associated with increased intracellular glycogen stores. For example, glycogen increase in G1-phase had been reported for some colon cancer cell lines [33]. This possibility is unlikely because treated A549 cells accumulate glycogen but do not demonstrate complete growth arrest as evidenced by FACS analysis. Incomplete growth arrest results in slow growth with increased glycogen and is similar to untreated slow growing cells such as astrocytes and HSF55 cells, which had significantly higher basal glycogen content than more rapidly growing cells such as C6 and T98G gliomas. This inverse relationship between glycogen content and growth had also been described with several intestine carcinoma cell lines [34]. Thus in some cases of growth inhibition described here, CP-91,149 does not cause complete growth arrest.

The precursor for glycogen formation is glucose-1-phosphate that is generated from glucose-6-phosphate, a central metabolite for glycolysis and the pentose-phosphate cycle. Glucose-6-phosphate is, therefore, important as precursor for energy production and nucleotide synthesis. Glycogen formation could restrict intracellular glucose and contribute to a shortage of energy and metabolites for growth with the observed slower growth and growth arrest. This would suggest that part of the glucose-6-phosphate is converted to glycogen and degraded by GP to glucose-1-phosphate, which is converted to glucose-6-phosphate. Since GP is regulated by phosphorylation and various allosteric effector [4] inhibition or activation of GP could represent a mechanism to regulate cell proliferation.

The GP isoform important for cell proliferation appears to be brain GP, which we demonstrated to be present in all 59 cell lines. Brain GP was also found to be expressed in preneoplastic gastric and colon cancers, which confirms its importance for proliferation [10]. Surprisingly, we observed that the brain GP protein level is low in a majority of the 59 cell lines. One explanation could be that the reduced levels of total brain GP may be sufficient to support growth, since we also found that all cells with increased GP activity showed reduced expression of brain GP, reflecting a high GP-a (activated form)/GP-b (inactive form) ratio (Fig. 5). Cell lines such as transformed fibroblasts, C6 and U87 gliomas with low brain GP expression were resistant to CP-91,149 and did not respond with glycogen accumulation and growth inhibition. One likely possibility for their drug resistance is an increase in the IC_{50} value for CP-91,149 in GP inhibition in these cells. CP-91,149 stabilizes the less active T-state of GP and can cause dephosphorylation of GP-a. Furthermore, effector molecules like glucose, which allosterically regulate GP activity, decrease the IC_{50} value for CP-91,149 in GP inhibition [18,19] so that the intracellular levels of positive or negative effector molecules and the phosphorylation state of GP likely contribute to the efficacy of CP-91,149 to inhibit GP.

Inhibition of cell growth by CP-91,149 appears to be dependent upon levels of the brain GP protein and that this is associated with intracellular glycogen accumulation, suggesting the possibility that expression of brain GP is a phenotypic manifestation of other regulatory gene alterations. As an example, breast cancer cells express lower brain GP levels when they overexpress the transfected Her2/Neu oncogene as compared with transfected cells having lower levels of the Her2/Neu gene product [11]. Potentially, downregulation of brain GP by an upstream element may result in ablation of the regulatory function of GP. However, using correlation analyses to search for targets in the NCI database that were inversely expressed was unsuccessful. We postulate that there may not be a single protein in all cell lines but

cancer type specific proteins responsible for the down-regulation of GP protein. We found two proteins, which are coordinately expressed with brain GP, ADP-ribosyltransferase 3 (ART3) and colony stimulating factor 2 receptor α (CSF-R α). Although there is no obvious connection of both proteins to brain GP, CSF-R α is involved in upregulation of glucose uptake via the Pi3 kinase pathway [35]. Further, the disruption of a protein associated with CSF-R α, GRAP, in the yeast *Saccharomyces cerevisiae* leads to a stress response manifested by early glycogen accumulation [36]. This suggests that CSF-R α maybe involved in the regulation of glycogen metabolism.

Acknowledgments

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