

Expression Profiling of Homocysteine Junction Enzymes in the NCI60 Panel of Human Cancer Cell Lines

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Abstract

Methionine metabolism provides two key cellular reagents: S-adenosylmethionine and glutathione, derived from the common intermediate, homocysteine. A majority of cancer cells exhibit a methionine-dependent phenotype whereby they are unable to grow in medium in which methionine is replaced by its precursor, homocysteine. Additionally, CpG island hypermethylation of tumor suppressor gene promoters is observed in a background of global hypomethylation in cancerous cells. In this study, we have profiled the expression levels of the homocysteine junction enzymes, methionine synthase (MS), MS reductase (MSR), and cystathionine β -synthase (CBS) in the NCI60 panel of cancer cell lines. The doubling time of non-small lung cell cancer lines, which exhibit the lowest levels of MS within the panel, was significantly correlated with expression of MS. The ratio of MS to MSR varied over a 5-fold range in the different cell types, which may modulate methionine synthesis. Interestingly, markedly reduced CBS expression was seen in the methionine-dependent prostate cancer cell line, PC-3, but not in the methionine-independent cell line, DU-145. However, neither provision of the transsulfuration pathway product, cysteine, nor overexpression of CBS rescued the growth impairment, indicating that reduced CBS was not responsible for the methionine-dependent phenotype in this cell line. (Cancer Res 2005; 65(4): 1554-60)

Introduction

Multiple aberrations in methyl group metabolism are observed in human cancers ranging from the methionine-dependent phenotype of a majority of cancer cell lines to alterations in epigenetic methylation patterns (1, 2). The methionine-dependent phenotype refers to the inability of neoplastic cells to grow in a folate-replete medium in which methionine is replaced with homocysteine (3). The latter is a substrate for the reaction catalyzed by methionine synthase (MS), which transfers the methyl group from methyltetrahydrofolate to homocysteine to regenerate methionine (Fig. 1). At a different level, cancer cells exhibit perturbations in methylation metabolism as evidenced by dimorphic changes in genomic methylation patterns: global hypomethylation, which directly affects chromosomal stability, is accompanied by hypermethylation in CpG islands (1, 4). For instance, patients with immunodeficiency-centromeric instability-

facial anomalies syndrome who have mutations in DNA methyltransferase 3b exhibit specific decondensation of the centromeric and pericentromeric regions of the chromosome that is associated with hypomethylation in these regions (5). In contrast, aberrant promoter methylation leads to epigenetic silencing of tumor suppressor genes, viz. breast cancer 1 early onset (6) and von Hippel-Lindau syndrome (7) genes.

Methionine is an essential amino acid that is converted in the methionine cycle to the important cellular methylating agent, S-adenosylmethionine (Fig. 1, *AdoMet*). Homocysteine is a junction metabolite in this pathway and can either be salvaged to the methionine cycle by a transmethylation reaction or be converted to cysteine via the transsulfuration pathway. Cysteine is used in protein biosynthesis and in the generation of glutathione, a major cellular antioxidant. In addition to being a key methyl donor, S-adenosylmethionine plays an important allosteric role in regulating sulfur metabolism in mammals by enhancing flux of homocysteine through the transsulfuration pathway when its concentrations are high, and in activating transmethylation when its concentrations are low and methionine is limiting (8).

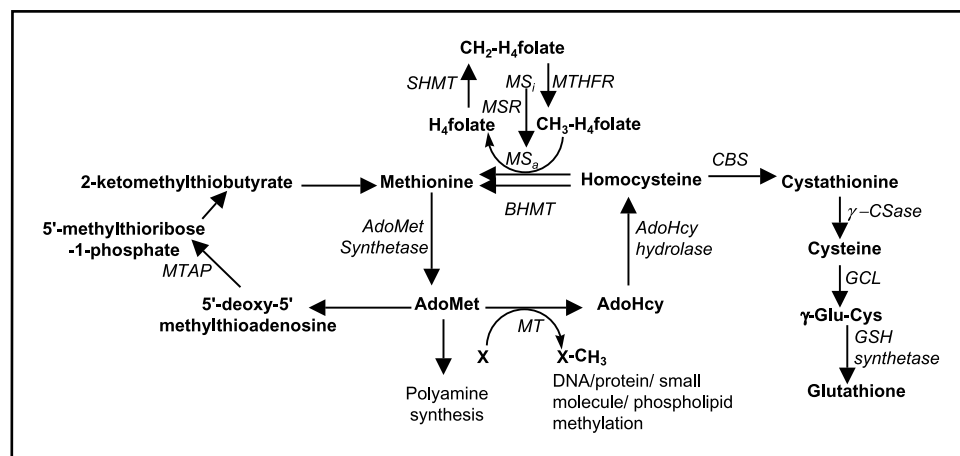
Although the molecular basis of the methionine-dependent phenotype of cancer cells is unknown, it has resulted in the development of methioninase as a potential antineoplastic agent (9). The inability of methionine-dependent tumors to survive in Met⁻Hcy⁺ medium suggests MS as a prime candidate for harboring mutations responsible for the phenotype. However, mutations in the coding sequence of MS have not been found in the limited number of tumor cell lines that have been analyzed (10). In addition, defects in the methionine salvage enzyme, methylthioadenosine phosphorylase, although often associated with methionine-dependent tumor cell growth, do not seem to be responsible for this phenotype (10).

Polymorphisms in enzymes involved in methionine metabolism, viz. MS (11), methylenetetrahydrofolate reductase (12, 13), and cystathionine β -synthase (CBS; ref. 14), have been described that are quite prevalent in the general population. Differences in the association between germ-line variants of methylenetetrahydrofolate reductase and MS and the degree of global genomic hypomethylation and CpG island hypermethylation of tumor suppressor genes have been reported (15). Furthermore, correlations between the common polymorphisms in enzymes involved in methionine metabolism and the incidence of specific types of cancers are being actively explored (16–19). Methionine metabolism is dependent on a number of cofactors, notably the B vitamins, folic acid, B₁₂, and B₆. Thus, the observed allelic differences in DNA methylation susceptibility suggest a plausible mechanism for modulation of cancer risk by interactions between the genotype of an individual and environmental/nutritional factors.

Note: W. Zhang and A. Braun contributed equally to this work.

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Figure 1. An overview of methionine metabolism. The enzymes discussed in this study are noted. MS_i and MS_a refer to the inactive and active forms of MS.



In principle, the methionine-dependent phenotype may result from (i) decreased resynthesis of methionine (via the methionine cycle or the salvage pathway), (ii) increased demand for methionine in rapidly growing cells that is not met by the recycle pathways, or (iii) increased demand for cysteine, which is derived from methionine and is the limiting reagent in the biosynthesis of glutathione of which concentrations range from 1 to 10 mmol/L depending on the tissue. In the third instance, methionine dependence would result if homocysteine transport from the medium is insufficient to maintain the transsulfuration flux. The key junction enzymes which determine the competing flux between the transmethylation and transsulfuration branches of the methionine metabolic pathway are MS and CBS. In addition, the activity of human MS is critically dependent on the presence of a recently discovered auxiliary redox protein, MS reductase (MSR; ref. 20). Whereas the expression level and the coding sequence of MS have been analyzed in 12 cancer cell lines (10), the levels of MSR and CBS have not been reported previously. In this study, we have profiled the relative expression levels of these three enzymes in a more extensive panel of 60 cancer cell lines that includes lung, breast, ovary, prostate, kidney, colorectal, and central nervous system cancers, as well as leukemias and melanomas.

Materials and Methods

Cell Lines. Cell lines were obtained from the National Cancer Institute Developmental Therapeutics Program (NCI60 cell line panel), shipped on dry ice as frozen pellets ($\sim 10^6$ cells each). The morphologic and immunocytochemical properties and methods of handling the cell lines used in this study have been previously described (21). For preparation of cellular extracts, the cell pellets were thawed and resuspended in 0.5 mL PBS containing Complete™ protease inhibitor tablets (Roche, Indianapolis, IN). The cell suspensions were sonicated on ice (six 5-second bursts with 30-second pause intervals at a power setting of 4), using a Misonix Sonicator XL2020 (Misonix, Inc., Farmingdale, NY), and then spun for 20 minutes in a refrigerated Beckman tabletop centrifuge (4°C, 14,000×g) to obtain S9 supernatant. The resulting supernatants, containing a mixture of cytosolic and microsomal proteins (22), were aliquoted and stored at -70°C. Total protein concentration of S9 supernatants was measured by the Bradford assay (Bio-Rad, Hercules, CA).

Western Blot Analysis. Denaturing SDS-PAGE and chemiluminescence-based Western blot analysis were used to determine relative amounts of MS, MSR, cytochrome P450 reductase, and CBS protein in S9 supernatants from each cell line. SDS-PAGE was done according to the method of Laemmli (23). Fifty micrograms of protein were applied to each lane and an equal

amount of HepG2 cell extract was used as an internal control in each gel. Prestained molecular weight protein standards were obtained from Bio-Rad. For immunoblotting, proteins were transferred onto polyvinylidene difluoride membranes (Bio-Rad) and blocked for 5 hours at room temperature in TTBS [50 mmol/L Tris-HCl (pH 7.5), 0.5 mol/L NaCl, 0.05% v/v Tween 20] with 5% nonfat milk. After washing several times with TTBS, blots were incubated with the appropriate primary antibodies dilutions in TTBS, 5% nonfat dry milk, overnight at 4°C. The membranes were washed in TTBS and the secondary antibody, alkaline phosphatase-labeled anti-rabbit antibody (Sigma, St. Louis, MO), was applied at a dilution of 1:5,000 (in TTBS, 5% nonfat dry milk) for 1 hour at room temperature. The binding of the primary antibody was detected using a chemiluminescence detection system (CDP-Star, Sigma). Protein band intensity was quantified by densitometry using a GelDoc system (Bio-Rad). Actin was used as an equal loading control. Briefly, polyvinylidene difluoride membranes were stripped by incubating in glycine buffer (pH 2.0) for 60 minutes, washed in TTBS, and incubated with anti-actin antibodies (Sigma), according to the protocol of the vendor. The blots were rinsed in TTBS and developed using the same chemiluminescence assay as described above. Samples for each cell line were run in duplicate in at least three separate experiments. The antibodies for MS, MSR, and CBS were generated in-house and used as described previously (20, 24, 25). The antibodies for P450 reductase were a generous gift from Dr. Bettie Sue Masters (The University of Texas Health Science Center, San Antonio, TX).

Cell Culture. PC-3, DU-145, and HepG2 (purchased from American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 containing 100 μmol/L methionine (Met⁺) or in RPMI 1640 in which methionine was replaced with 200 μmol/L homocysteine (Met⁻Hcy⁺). Cells were counted using a hemocytometer. To examine the effect of cysteine on the methionine-dependent PC-3 cell line, cells were cultured in Met⁻Hcy⁻ supplemented with 200 μmol/L *N*-acetylcysteine and the number of cells was counted on days 0, 2, 4, and 6. Each experiment was done in duplicate.

Data Analysis. Most statistical analyses were carried out using the *R* statistical package, Perl, and Microsoft Excel. The Pearson correlation coefficients between the four proteins in this study and their mRNA levels were calculated in all cell lines and in each of the tumor panels using Eq. A:

$$cc = \frac{n \sum xy - (\sum x)(\sum y)}{\sqrt{n(\sum x^2) - (\sum x)^2} \sqrt{n(\sum y^2) - (\sum y)^2}} \quad [A]$$

where *cc* represents the strength of the linear relationship between the paired *x* and *y* values in the sample, and *n* represents the number of pairs of *x* and *y* (i.e., the number of cell lines).

Significance (at the $P < 0.05$ and $P < 0.01$ levels) was examined with the two-tailed t test using Eq. B.

$$t = \frac{cc}{(1 - cc^2)/(n - 2)} \quad [B]$$

The microarray data, the target activities, and the doubling times of the NCI60 set were downloaded from the website of the Developmental Therapeutics Program of the National Cancer Institute <http://www.dtp.nci.nih.gov>. The differentiation levels of NCI60 cell lines are reported in ref. 26 and the methionine dependencies of the cancer cell lines are reported in refs. 3, 27, 28. The correlative analysis between protein expression levels and drug sensitivities was done by Dr. Susan Holbeck (National Cancer Institute) using the program COMPARE and the databases developed by the NCI developmental therapeutics program at the following URL: (http://dtp.nci.nih.gov/docs/cancer/searches/cancer_open_compounds.html).

Results

Expression of Methionine Synthase and Methionine Synthase Reductase in the NCI60 Panel. The expression levels of MS and its redox partner protein, MSR, were examined in the NCI60 panel relative to their expression in HepG2 cells (Fig. 2). The expression of MS varied over a 9-fold range in the different cell lines, being lowest in non-small cell lung cancer cells and highest in melanomas (Table 1). Interestingly, a significant and positive correlation between MS expression and doubling time ($r = 0.745$, $P < 0.05$) was only observed in the non-small cell lung cancer cell lines, which expressed the lowest levels of MS compared with all other cell types evaluated in this study (data not shown). The expressions of MS and MSR at the protein level were poorly correlated to the mRNA expression level for each ($r = 0.3$, $P < 0.05$), suggesting a role for translational regulation (Fig. 3). Indeed, translation regulation of MS has been reported (29).

The expression of MSR varied over a narrower 2.7-fold range with the highest levels found in melanomas and the lowest in ovarian cells (Table 1). It was also low in CNS, colon, prostate, and non-small cell lung cancer lines. The ratio of MS to MSR varied over a 5-fold range with the ratio being least favorable in ovarian and melanoma cells.

The methionine-dependent phenotype of only a few of the cell lines in the NCI60 panel is known. These include A498 (renal), PC-3 (prostate), MCF7 (breast), SW-620 (colon), HL-60 (leukemia), K-562 (leukemia), and A549 (non-small cell lung carcinoma; refs. 3, 27, 28, 30). MS and MSR were expressed in all seven of these cell lines at levels that were comparable to those in other cell lines originating from the same tissue. In fact, expression of both MS and MSR was observed in all cell lines, consistent with the observation that MS is an essential gene (31). Only two of the cell lines used in this study, HepG2 (liver) and DU-145 (prostate), are described in the literature as being methionine-independent.

Expression of Cystathionine β -Synthase in the NCI60 Panel of Cancer Cells. Expression of CBS in different cancer cell lines was evaluated relative to its levels in HepG2 cells (Fig. 2). CBS expression levels varied over a 4-fold range being highest in breast cancer cells and lowest in melanomas and non-small cell lung cancers (Fig. 2). CBS could not be detected in colon and leukemia cells (Table 1). The expression of CBS at the protein level was moderately well correlated to its mRNA expression level ($r = 0.66$, $P < 0.01$, Fig. 3).

Methionine Dependence of Prostate Cancer Cell Lines. Due to two prostate cancer cell lines, DU-145 and PC-3, having been described as being methionine-independent and methionine-dependent, respectively (32), a direct comparison of the expression levels of the proteins monitored in this study could be made. First, the growth phenotype was confirmed by comparing the growth of the cell lines in Met^+ versus $\text{Met}^- \text{Hcy}^+$ medium (Fig. 4A). DU-145, like HepG2 cells (not shown), exhibited only slightly reduced growth in the absence of methionine in contrast to PC-3, which displayed significantly reduced growth. Of the enzymes examined in this study, a marked difference was observed only in CBS levels in the two prostate cancer cell lines (Fig. 2). Thus, the methionine-dependent cell line, PC-3, exhibited ~ 16 -fold lower CBS than the methionine-independent prostate cancer line, DU-145.

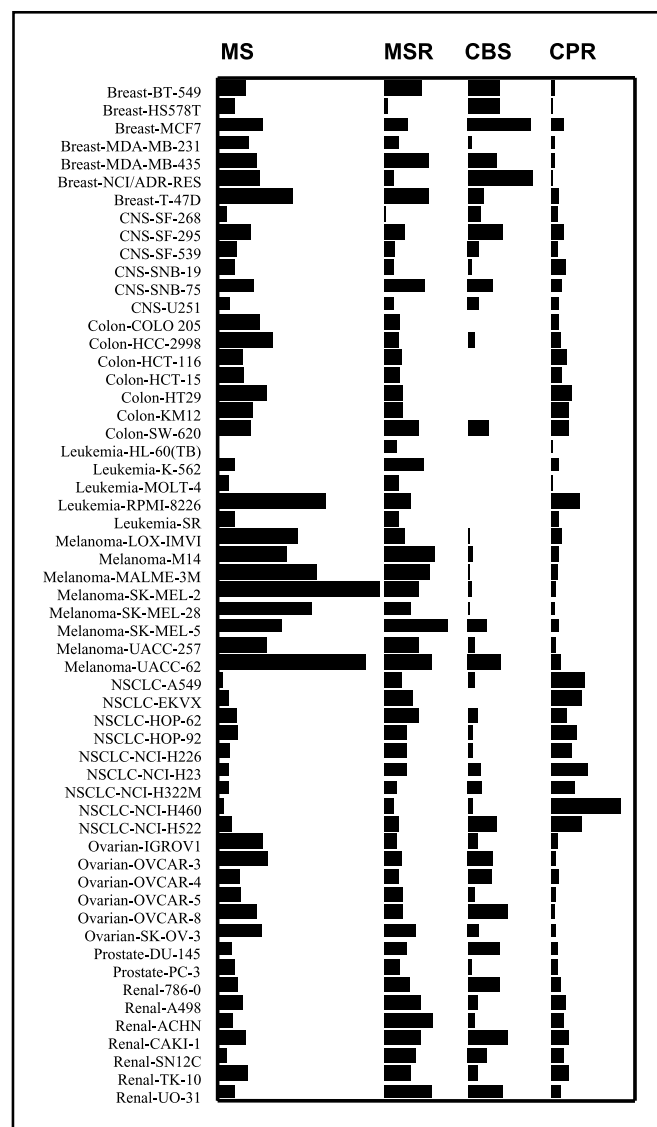


Figure 2. Comparison of expression levels of homocysteine junction enzymes in the NCI60 panel. The protein levels were quantitated from Western blots as described under Materials and Methods and expressed as a ratio of the expression level of the same protein in HepG2 cells. Equal loading was monitored by immunodetection of actin in each blot as described under Materials and Methods. CPR, MSR, and CBS denote cytochrome P450 reductase, methionine synthase reductase, and CBS, respectively.

Table 1. Expression levels of homocysteine junction enzymes and cytochrome P450 reductase in cancer cell lines

	MS	MSR	CBS	CPR
Breast	1.42 ± 0.67	0.88 ± 0.61	1.20 ± 0.83	0.16 ± 0.16
CNS	0.75 ± 0.39	0.54 ± 0.49	0.55 ± 0.42	0.33 ± 0.12
Colon	1.35 ± 0.41	0.68 ± 0.24	0.13 ± 0.27	0.50 ± 0.17
Leukemia	1.38 ± 1.69	0.76 ± 0.42	N.D.*	0.32 ± 0.39
Lung	0.42 ± 0.19	0.71 ± 0.28	0.32 ± 0.30	1.12 ± 0.56
Melanoma	3.43 ± 1.44	1.43 ± 0.49	0.29 ± 0.41	0.23 ± 0.10
Ovarian	1.33 ± 0.43	0.65 ± 0.23	0.66 ± 0.45	0.17 ± 0.05
Prostate	0.55 ± 0.07	0.68 ± 0.16	0.60 ± 0.75	0.22 ± 0.02
Renal	0.72 ± 0.26	1.28 ± 0.33	0.74 ± 0.49	0.46 ± 0.13
Average	1.33 ± 1.19	0.88 ± 0.48	0.54 ± 0.56	0.44 ± 0.41

NOTE: Expression of each of the enzymes, CBS (cystathionine b-synthase), CPR (cytochrome P450 reductase), MS (methionine synthase), and MSR (methionine synthase reductase), is given relative to the level of each enzyme in HepG2 cells.

*N.D., not detectable.

In principle, lower CBS expression in PC-3 cells could result in methionine dependence by restricting the supply of cysteine derived from methionine via the transsulfuration pathway. This assumes, however, that transport of homocysteine from the Met⁻Hcy⁺ medium into cells is insufficient to meet demands, a hypothesis that is consistent with the ability of low concentrations of methionine (1 μmol/L) to enhance utilization of extracellular homocysteine (33). To examine this possibility, the growth of PC-3 cells in Met⁻Hcy⁺ medium supplemented with cysteine derivative *N*-acetylcysteine was examined (Fig. 4B). However, growth was unaffected by provision of *N*-acetylcysteine.

The relation between reduced CBS expression and the methionine dependence of the PC-3 cell line was further explored by comparing the growth rates of derivative cell lines stably transfected with CBS or with the empty vector, pCMV3B. Overexpression of CBS in the stably transfected PC-3 cell lines was confirmed by Western blot analysis (not shown). However, a growth advantage in Met⁻Hcy⁺ medium was not conferred by overexpression of CBS (Fig. 4C). Together, these experiments reveal that whereas reduced CBS expression is associated with methionine dependence in the PC-3 cell line, it is not causal.

Expression of Cytochrome P450 Reductase. Of the four enzymes investigated in this study, the expression level of cytochrome P450 reductase varied the least between different cell lines (Fig. 2). The one exception was non-small cell lung cancer lines in which cytochrome P450 reductase was expressed at a significantly higher level (Table 1). The reason for including cytochrome P450 reductase in this study was to permit comparison of its expression levels relative to that of MSR. Recently, a homologue of MSR, human novel reductase 1 (NR1), has been described whose physiologic function is unknown (34). However, like MSR and cytochrome P450 reductase, NR1 can transfer electrons to a variety of small molecule acceptors and could potentially be involved in redox activation or inactivation of drugs or in superoxide generation. The level of NR1 relative to cytochrome P450 reductase has been reported to be elevated in cancer cells although the significance of this is unknown (34). Due to the similarities in the properties of MSR and NR1, which extend to their cytoplasmic localization (versus microsomal for P450 reductase), we were interested in evaluating whether the

abundance of MSR is increased relative to P450 reductase in cancer cells.

Relative to the ratio of MSR to cytochrome P450 reductase in HepG2 cells (arbitrarily set at 1), the ratio was increased >2-fold in

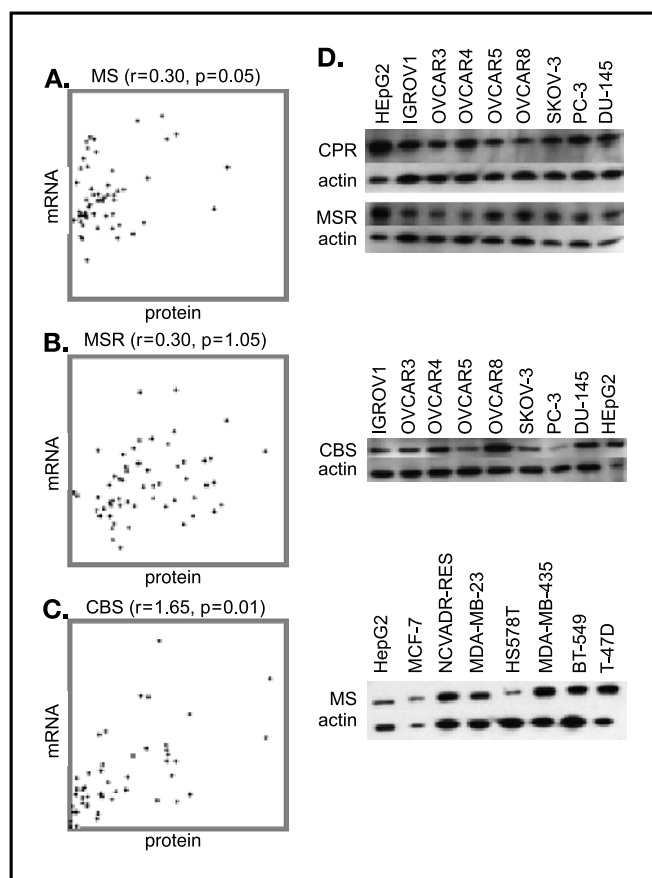


Figure 3. Correlations between mRNA and protein expression levels in the NCI60 panel. The mRNA levels were obtained from a microarray database at the NCI website (<http://dtp.nci.nih.gov/mtargets/download.html>) and compared with the protein expression data set generated in this study. Correlations are shown for CBS (A), MS (B), and MSR (C). D, representative Western blots of the four enzymes and actin used as an equal loading control.

Table 2. Correlative analysis of MS and MSR expression profiles and drug responses

Target	Compound	P (two-tailed)	Pearson correlation coefficient*	Possible mechanism
Whole database				
MS	628567	4.7×10^{-7}	0.67	Not known
	631075	1.1×10^{-6}	0.706	Not known
	677595	1.7×10^{-6}	0.618	Not known
	658473	1.8×10^{-6}	0.622	Not known
MSR	646610	3.6×10^{-7}	-0.664	Not known
	646608	5.3×10^{-7}	-0.657	Not known
BEC database				
MS	Chloroestruxin	4.9×10^{-6}	0.577	ATPase
	Strophanthoside K	1.6×10^{-5}	-0.542	Cardiac glycoside
MSR	646610	3.6×10^{-7}	-0.664	Not known
	646608	5.3×10^{-7}	-0.657	Not known
	646607	1.0×10^{-5}	-0.596	Not known
	646609	1.6×10^{-5}	-0.585	Not known

*A positive correlation indicates that higher target levels make the cells more sensitive to the particular drug whereas a negative correlation implies the opposite.

the majority of cells in the NCI60 panel, being highest in melanoma cells (>6-fold higher) followed by breast > prostate > kidney \approx ovary > leukemia cells. The expression ratio in CNS, colon, and non-small cell lung cancer cells was \sim similar to that in HepG2 cells.

Correlation between Expression Profiles and Drug Sensitivity. The expression profiles of MS, MSR, and CBS were subjected to a correlative analysis with the mean graph pattern of drug response at the GI50 (50% growth inhibition) level of effect using the NCI Developmental Therapeutics Program databases (Table 2). When the open database (comprising \sim 30,000 compounds) was queried, the expression pattern of MS correlated positively (i.e., higher MS enhances drug sensitivity), but weakly, with four compounds, 628567, 631075, 677595, and 658473, of which mechanisms of action are not known. In contrast, MSR expression correlated negatively (i.e., higher expression increases drug resistance), but weakly, to two compounds, 646610 and 646608, of which modes of action are also not known. Expression of CBS expression was not significantly correlated (not shown). In the BEC-selected database comprising \sim 3,000 compounds, MS correlated positively with chloroestruxin and negatively with strophanthoside K, whereas MSR expression was negatively correlated with 646607 and 646609, in addition to the two compounds found in the open database. No significant correlations were found between CBS expression levels and drug sensitivity. In the standard agent database, containing 170 compounds that have been developed clinically, no significant correlations were found with the expression levels of any of the three proteins.

Discussion

The changes that lead to altered methylation metabolism in cancer cells are not understood and enzymes involved in the complex metabolic cycle that generates S-adenosylmethionine, the universal methyl donor, are good candidates in the search for correlated changes. An additional cancer-correlated phenotype that could be associated with this metabolic pathway is the impeded growth that most cancer cells display when methionine is

replaced by its immediate metabolic precursor, homocysteine (35, 36). This tumor-associated methionine-dependent phenotype has also been observed *in vivo* and has been used to explain the antitumor mechanism of *cis*-diammine dichloroplatinum (37). Given the methionine dependence of a large number of cancer cell lines, methionine restriction has been considered as a potential cancer therapeutic strategy, but the biochemical mechanism of methionine dependence remains unclear (38).

Homocysteine is an intermediate in methionine metabolism and connects the biosynthesis of cysteine to methionine (Fig. 1). In principle, aberrations in methyl group metabolism associated with cancer cells can result from dysfunction of any of the three homocysteine junction enzymes, MS, MSR, or CBS. A number of recent studies have examined the association between the risk for specific cancers and common polymorphisms in these three enzymes (16–19). However, the relative protein expression profiles for these enzymes and their variations, if any, in different cancer cell lines have not been reported. In this study, we have done a comprehensive analysis of three proteins involved in methionine metabolism and of cytochrome P450 reductase in a panel of representative human cancer cell types.

Since 1990, the National Cancer Institute Developmental Therapeutics Program has screened >100,000 chemical compounds for anticancer activity using the NCI60 cell panel. The latter has also been extensively profiled at the DNA, RNA, protein, and functional levels for correlation with pharmacologic sensitivity of the individual cell lines. Recently, Weinstein et al. (39) reported the expression profiles of 52 proteins in this panel. In this study, we have employed the NCI60 panel to profile the expression levels of methionine metabolism enzymes located at the homocysteine metabolic junction.

Our study reveals that no single aberration in MS, MSR, or CBS expression level is correlated with cancer or with the methionine-dependent phenotype of cancer cell lines. However, three limitations of our study deserve discussion. First, we have not measured enzyme activities for the enzymes under study in the NCI60 panel of cells. The relative insensitivity of the assays

for these enzymes rendered this approach impracticable. Second, expression profiles for the enzymes have been compared across the panel rather than for pairs of normal versus cancerous tissues. Finally, the growth phenotype in Met^+Hcy^- medium is only known for a subset of the cells in the NCI60 panel.

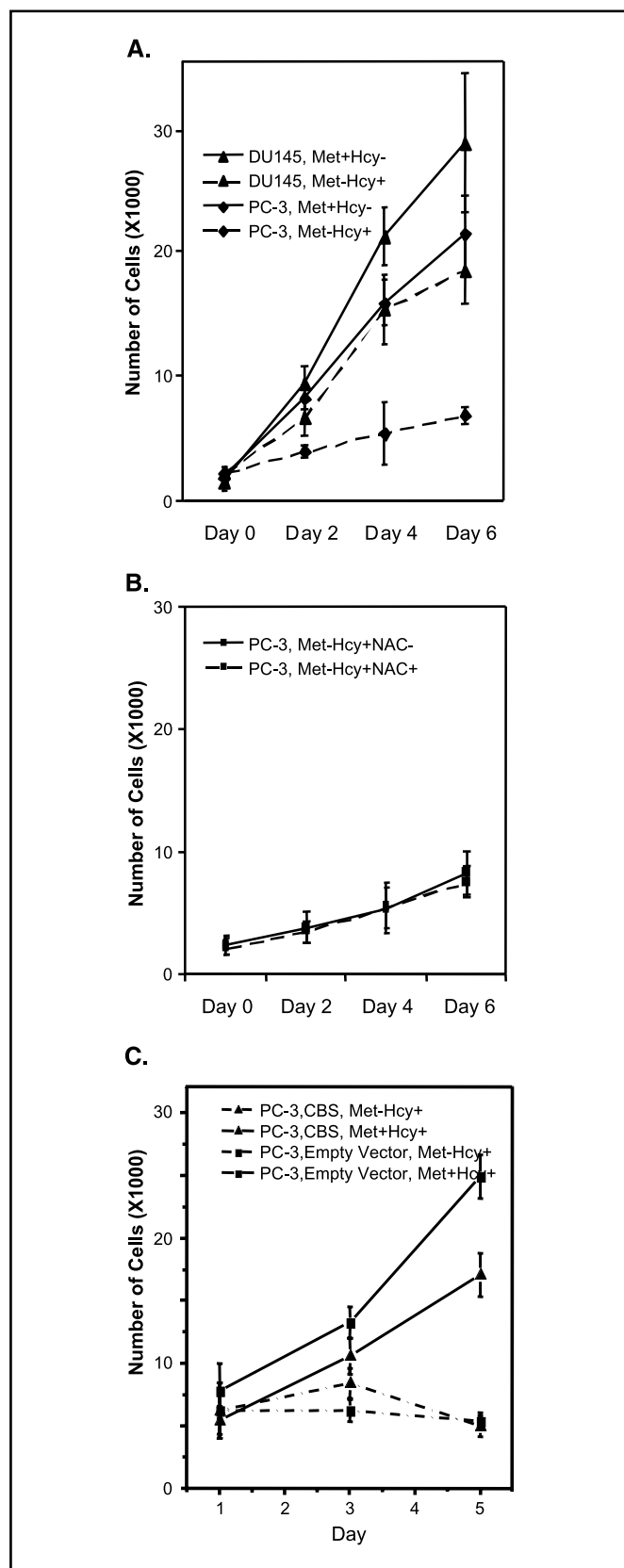
A pair of prostate cancer cell lines in the NCI60 panel, PC-3 and DU-145, displays contrasting phenotypes with respect to growth on methionine. Interestingly, whereas significantly reduced CBS expression is associated with methionine dependence in the prostate carcinoma cell line, PC-3, provision of the transsulfuration product, cysteine, or overexpression of CBS did not correct the growth impairment (Fig. 4). Thus, the association of the methionine-dependent phenotype with reduced CBS is unlikely to be responsible for the phenotype.

In the limited number of transformed cell lines in which MS activity has been measured (33, 40, 41) or the coding sequence of MS has been analyzed (10), a discernable defect has not been observed. Comparable MS activity in the presence of exogenous methylcobalamin was also reported in human melanoma cell line variants, which exhibited methionine-dependent (Me-Wo-LC1) and methionine-independent (D3.12.2) phenotypes (42). However, Me-Wo-LC1 showed enhanced efflux of cobalamin and very low intracellular concentration of methylcobalamin, the active cofactor form of MS, relative to D3.12.2. A reduction in total intracellular cobalamin concentration is inconsistent with the reported normal MS activity in the methionine-dependent melanoma, owing to the apoenzyme being unstable and prone to degradation (43). Furthermore, we have not observed reduced MS expression and, in fact, expression levels of this enzyme in melanoma cells are among the highest in the NCI60 panel (Table 1 and Fig. 2). Alternatively, the lower methylcobalamin concentration in methionine-dependent Me-Wo-LC1 could have resulted from reduced activity of MSR, which converts oxidized cobalamin bound to MS to methylcobalamin (20). The MS/MSR ratio varies in the NCI60 panel and is unfavorable particularly in several melanoma lines where MS levels are high. However, in the pair of prostate carcinoma lines DU-145 and PC-3 displaying differences in methionine auxotrophy, the ratio is comparable.

Folate cycle enzymes such as thymidylate synthase and dihydrofolate reductase have been successfully targeted for cancer chemotherapy for many years (44). Despite the large number of antifolates that have been tested, specific inhibitors of MS, long considered to be a choice chemotherapeutic target, have eluded discovery. The correlations between MS expression and four compounds in the NCI Developmental Therapeutics Program databases identified in Table 2 are weak. Nevertheless, these compounds could potentially find utility as lead templates for modification in targeted drug design.

In summary, this study provides the first comprehensive analysis of expression profiles of the three homocysteine junction enzymes involved in methionine metabolism in a representative set of 60 human cancers. We focused on obtaining protein expression

Figure 4. The effect of *N*-acetylcysteine and CBS overexpression on the methionine dependence of prostate cancer cell lines. **A**, growths of PC-3 and DU-145 were compared in Met^+ and Met^+Hcy^- medium. **B**, addition of $200\ \mu\text{mol/L}$ *N*-acetylcysteine to Met^+Hcy^- medium failed to rescue the methionine-dependent phenotype of DU-145 cells. **C**, comparison of growth rates of PC-3 cells stably transfected with the empty expression vector, pCMV3B, or same vector containing full-length myc-tagged human CBS.



profiles owing to the studies from our laboratory (24, 29)¹ having revealed they are regulated at the translational level, which limits the utility of the existing mRNA profiles of these genes. Our study provided insights into tissue specific variations in the expression of the three enzymes, which could be particularly useful in correlative analyses of polymorphic variants in these enzymes and specific types of cancers. The protein level of MS, an essential enzyme, is lowest in non-small cell lung cancer cells and is significantly correlated with the doubling time of these cell lines. The study also reveals varying ratios of MS to MSR expression, which is expected to modulate the efficacy of the transmethylation reaction catalyzed by MS and therefore

subsistence in a Met⁻Hcy⁺ medium. A limited number of compounds screened as potential anticancer drugs are weakly correlated with the expression of MS and MSR. Finally, the association between greatly reduced expression of CBS and the methionine dependence of PC-3 does not underlie the nutritional auxotrophy of this prostate cancer cell line.

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¹ Prudova and Banerjee, unpublished data.

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