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Association of transcriptional levels of folate-mediated one-carbon metabolism-related genes in cancer cell lines with drug treatment response

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Abstract

Folate-mediated one-carbon metabolism is essential for growth and survival of cancer cells. We investigated whether the response of cancer cells to antitumor treatment may be partially influenced by variation in expression of one-carbon metabolism genes. We used cancer cell line information from the Cancer Cell Line Encyclopedia and the Genomics of Drug Sensitivity in Cancer resources to examine whether variation in pretreatment expression of one-carbon metabolism-related genes was associated with response to treatment. *GART, TYMS, SHMT2, MTR, ALDH2, BHMT, MAT2B, MTHFD2, NNMT*, and *SLC46A1* showed modest statistically significant correlations with response to a variety of antitumor agents. Higher expression levels of *SLC46A1* were associated with resistance to multiple agents, whereas elevated expression of *GART, TYMS, SHMT2, MTR, BHMT*, and *MAT2B* was associated with chemosensitivity to multiple drugs. *NNMT* expression was bimodally distributed and showed different directions of association with various agents. Correlation of increased *NNMT* expression with sensitivity to dasatinib was validated in

Abbreviations: 10-formylTHF, 10-formyltetrahydrofolate; 5,10-methylene-THF, 5,10-methylenethetrahydrofolate; AHCY, adenosylhomocysteinase; ALDH1L1, aldehyde dehydrogenase 1 family, member L1; ALDH2, aldehyde dehydrogenase 2 family; ALK, anaplastic lymphoma kinase; ALL, acute lymphoblastic leukemia; AMT, aminomethyltransferase; ATF4, activating transcription factor 4; ATIC, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase; BHMT, betaine-homocysteine methyltransferase; CBS, cystathionine β-synthase; CCLE, Cancer Cell Line Encyclopedia; CTH, cystathionase; DDR1, discoidin domain receptor 1; DHF, dihydrofolate; DHFR, dihydrofolate reductase; EDNRA, endothelin-1 receptor (endothelin receptor type A); EGFR, epidermal growth factor receptor; FDR, false discovery rate; FOLH1, folate hydrolase 1; FOLR1, folate receptor 1; FOLR2, folate receptor 2; FOLR3, folate receptor 3; FTCD, formimidoyltransferase cyclodeaminase; GART, glycinamide ribonucleotide formyltransferase; GDSC, Genomics of Drug Sensitivity in Cancer; HDAC, histone deacetylase; HIF-1, hypoxia-inducible factor 1; LXR, liver X receptor; MAT1A, L-methionine S-adenosyltransferase 1, alpha; MAT2A, L-methionine S-adenosyltransferase 2, alpha; MAT2B, L-methionine S-adenosyltransferase 2, beta; MTHFD1, methylenetetrahydrofolate dehydrogenase 1; MTHFD2, methylenetetrahydrofolate dehydrogenase 2; MTHFD2L, methylenetetrahydrofolate dehydrogenase 2-like; MTHFR, 5, 10-methylenetetrahydrafolate reductase; MTHFS, methylenetetrahydrofolate synthase; mTOR, mammalian target of rapamycin; MTR, methionine synthase; MTRR, 5-methyltetrahydrafolate-homocysteine methyltransferase reductase; NCI, National Cancer Institute; NNMT, nicotinamide N-methyltransferase; OCM, one-carbon metabolism; OXPHOS, oxidative phosphorylation; PDK, pyruvate dehydrogenase kinase; PEMT, phosphatidylethanolamine-N-methyltransferase; PHGDH, phosphoglycerate dehydrogenase; PLK3, polo-like kinase 3; RIPK1, receptor-interacting serine/threonine-protein kinase 1; RMA, Robust Multiarray Average; ROS, reactive oxygen species; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT1, serine hydroxymethyl transferase 1; SHMT2, serine hydroxymethyl transferase 2; SLC19A1, solute carrier family 19 member 1; SLC46A1, solute carrier family 46 member 1; TCA, tricarboxylic acid; TCN2, transcobalamin 2; THF, tetrahydrofolate; TYMS, thymidylate synthase; VEGFR, vascular endothelial growth factor receptor.

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the NCI-60 cancer cell line panel. Pretreatment expression levels were correlated among many one-carbon metabolism genes. Expression of several folate genes was strongly associated with expression of multiple components of drug target pathways. Molecular mechanisms underlying associations of one-carbon metabolism gene with drug response require further investigation.

Keywords One-carbon metabolism, Folate, Cancer treatment, Drug sensitivity, Gene expression.

Introduction

Folate-mediated one-carbon metabolism (OCM), which involves folate and methionine cycles, regulates cellular nutrient status and redistribution of one-carbon units into reactions and processes that are vital for cellular functions [1,2]. One-carbon units generated from folic acid and amino acids are used for cellular biosynthesis, regulation of redox status, nucleic acid and protein methylation, and genome maintenance, which are necessary for cell growth and survival [1,3]. The folate cycle involves multiple biosynthetic reactions in the cytoplasm, mitochondria, and the nucleus [1,4,5]. These reactions lead to biosynthesis of critical components of cell function including synthesis of nucleotides, ATP and NADPH, and biosynthesis and interconversions of amino acids (e.g., serine, glycine, methionine, and histidine), and they also affect cellular methylation processes [6–8].

An ample amount of evidence has been accumulated that indicates that the genes involved in folate cycle reactions or folate transport play important roles in cancer risk and progression [1,8]. The OCM processes are critically important for the metabolic rewiring of cancer cells [2]. Numerous studies have documented that expression patterns and genetic variation of folate metabolism-related genes, dietary folate status, and changes in the folate cycle reactions and in DNA methylation, synthesis, and repair are associated with cancer risk and with *in vitro* and clinical response to drug treatment [4,8–14]. Inhibition of the components of the folate cycle can reduce growth and proliferation of malignant cells and increase DNA damage [15–17]. These mechanisms are suppressed by the action of anti-folate cancer drugs [6,15]. Increased levels of reactive oxygen species (ROS) under oncogenic transformation promote cancer cell proliferation with active cellular synthesis and methylation, but they may also lead to DNA damage-induced cell death if ROS levels are too high [16,17]. OCM reactions are critical for carcinogenesis, as they support increased proliferation of cancer cells by enabling high levels of cellular synthesis of critical molecular components, regulating redox reactions, and suppressing apoptosis by controlling the ROS levels [2,16,17].

Drug resistance of cancer cells may involve many biological processes, e.g. drug efflux, drug inactivation, drug target modification, DNA damage repair, and apoptosis inhibition [18]. A number of anticancer drugs may be activated and their targets could be modified by metabolic processes (18]. OCM reactions may affect drug efficacy through their effect on tumor growth and proliferation, their connection to other cellular metabolic reactions in the cell and to the processes of amino acid biosynthesis, nucleotide biosynthesis and DNA repair, and through their contribution to DNA and protein methylation processes which affect the expression of genes related to drug activation and drug targeting [1,8,13,19,20].

In an earlier study of five antitumor agents, we found an association between post-treatment expression changes of several genes involved in folate metabolism or transport with sensitivity or resistance to doxorubicin, paclitaxel, and cisplatin [21]. Given the importance of OCM reactions in growth and survival of cancer cells, we hypothesized that the response of cancer cells to drug treatment may be influenced by pretreatment levels of genes that are involved in folate transport and in OCM reactions. We examined whether pretreatment expression levels of some of the OCM genes were associated with sensitivity to a variety of antitumor agents, and whether expression levels of certain OCM genes could potentially be used as biomarkers associated with drug response. To study possible molecular mechanisms underlying such associations with drug response, we also examined whether pretreatment expression levels of OCM genes in tumor cells were correlated with each other and with expression levels of molecular components of drug target pathways.

Materials and methods

The datasets analyzed in this study are publicly available online from the Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC), and CellminerCDB resources [22–25].

Gene expression analysis

To study the relationship between pretreatment expression levels of OCM-related genes and cancer cell response to antitumor drugs, we used gene expression microarray data for 635 cell lines from the Cancer Cell Line Encyclopedia that were matched with drug response information for all agents available from the Genomics of Drug Sensitivity in Cancer dataset [22,25–27]. We further refer to this gene expression and drug response dataset as GDSC-CCLE dataset. The GDSC drug response data included the total of 251 drugs with sensitivity measures previously reported by Iorio et al. [27]. Based on earlier reports [1,3,4,8–10,14,28], we selected 34 OCM-related genes that included *AHCY, ALDH1L1, ALDH2, AMT, ATIC, BHMT, CBS, CTH, DHFR, FOLH1, FOLR1, FOLR2, FOLR3, FTCD, GART, MAT1A, MAT2A, MAT2B, MTHFD1, MTHFD2, MTHFD2L, MTHFR, MTHFS, MTR, MTRR, NNMT, PEMT, PHGDH, SHMT1, SHMT2, SLC19A1, SLC46A1, TCN2,* and *TYMS*. Their $\log_2$-transformed expression levels were downloaded from the from CCLE web resource of the Broad Institute [22]. These transcriptional measures had been generated using Affymetrix Human Genome U133 Plus 2.0 microarrays and normalized using the Robust Multi-array Average (RMA) algorithm [29]. For each gene, expression data from multiple microarray probes were averaged. The distribution of the $\log_2$-transformed gene expression measures was examined using histogram plots.

Drug sensitivity measures

To explore possible associations of pretreatment OCM gene expression with tumor cell response to a broad range of anti-cancer drugs, we examined correlations between pretreatment transcriptional levels of 34 OCM-related genes and drug sensitivity, measured as IC50 (the total drug inhibitor concentration that reduced cell activity by 50%) of 251 agents among all cancer cell lines [27]. Chemosensitivity values from the GDSC resource [25], in the ln(IC50) format, were obtained from the Supplementary Table 4A of Iorio et. el. [27] and converted to the $\log_{10}$(IC50) scale, to which we further refer as log(IC50). Fourteen agents (bicalutamide, UNC0638, JQ1, AZD6482, AZD6244, CHIR-99021, BMS-708163, GSK269962A, BMS-536924, RDEA119, GDC0941, olaparib, afatinib, and PLX4720) had duplicate IC50 measurements within the GDSC dataset. The concordance between duplicate cell line chemosensitivity measures within the GDSC dataset and their agreement with drug response measures independently generated by the CCLE and Genentech studies has been demonstrated and validated in previous studies [30,31]. Accordingly, all the 14 agents in the GDSC dataset had $p \leq 3.37 \times 10^{-8}$ for Pearson correlations between duplicate cell line response in our analysis. For these 14 agents with biological duplicates of drug response measures, we used a combined average of their log(IC50) drug response measurements from separate experiments [27].

Statistical analysis of association between OCM gene expression and drug response

Identities of the cell lines present in both CCLE and GDSC datasets were verified using information from Cellosaurus (32). Correlation and regression analyses were performed using R packages Hmisc, ggpubr, and lm.beta v.1.5–1, R environment v. 3.2.3, 3.4.1, and 3.5.3, RStudio v. 1.0.153, Python v. 2.7.12 and 2.7.15, and rpy2 v. 2.8.5. Association between pretreatment $\log_2$-transformed gene expression measures and log(IC50) of drug response was examined using Pearson correlation analysis. The resulting *p*-values were adjusted for multiple testing using the Benjamini and Hochberg's method [33] of false discovery rate (FDR) adjustment. FDR adjustment of correlations between OCM gene expression levels and log(IC50) values accounted for all 251 cancer drugs in the GDSC dataset and 34 folate genes. We selected genes associated with drug response using a cutoff of the absolute value of Pearson correlation coefficient $|r| > 0.3$ and $p < 0.05$ for FDR-adjusted *p*-values. The gene-drug pairs satisfying these criteria included 51 distinct agents, 50 of which had defined genetic drug targets (Table 1). We examined the biological roles of those OCM genes that satisfied these criteria and were associated with multiple antitumor agents, and of those cancer drugs which were associated with multiple genes. Analysis of overlapping agents associated with multiple folate genes was conducted using Venn diagrams inferred using Venny 2.1.0 [34].

To validate the robustness of the Pearson correlation results, we examined the non-parametric Spearman correlation of $\log_2$-transformed OCM gene expression values with log(IC50). The resulting *p*-values were adjusted for FDR while accounting for all 251 agents and 34 OCM genes. We examined which of the agents with Pearson $|r| > 0.3$ and FDR adjusted $p < 0.05$ in Pearson correlation also satisfied the threshold for the absolute value of Spearman correlation coefficient $|\rho| > 0.3$ and FDR adjusted $p < 0.05$ in Spearman correlation analysis.

Validation of selected top associations between gene expression and drug response in the NCI-60 dataset

After observing statistically significant associations (Table 1) between OCM gene expression and drug sensitivity in the 635 cell lines with available CCLE and GDSC measurements, we validated several strongest associations using the data from the NCI-60 cancer cell line panel. The 60 cell lines from that panel have been extensively characterized for their molecular features and screened manually for drug response at the U.S. National Cancer Institute (NCI) [35]. Microarray gene expression data (in the $\log_2$ format, representing an average of measures from five microarray platforms including Affymetrix HG-U95, HG-U133, HG-U133 Plus 2.0, and GeneChip Human Exon 1.0 ST arrays, and Agilent Whole Human Genome Oligo Microarray) were downloaded using the CellminerCDB resource v. 1.0 at the National Cancer Institute [24,36].

We identified the top 20 strongest associations in the CCLE-GDSC dataset with the highest values of Pearson $|r|$ listed in Table 1. These correlations involved 16 agents. For five of these agents, crizotinib, dasatinib, erlotinib, lapatinib, and methotrexate, drug response values, which had been generated through the NCI-60 screening by the NCI Developmental Therapeutics Program, were available for download from CellminerCDB. These NCI-60 data were downloaded from CellminerCDB in the format of *z*-scores computed from -$\log_{10}$(GI50), where GI50 is a molar concentration resulting in a 50% growth inhibition [24,36]. These *z*-scores were multiplied by -1 for comparability of the directions of associations with drug response between the GDSC-CCLE and NCI-60 datasets. We further refer to these transformed NCI-60 scores as the *z*-scores of log(GI50) values. Among the top 20 correlations listed in Table 1, GDSC sensitivity measures to 5 agents were associated with CCLE-measured expression of 6 OCM genes.

For each of these 6 drug-gene pairs, we selected the top 10% and the bottom 10% of the NCI-60 cell lines with the highest and the lowest expression levels of the OCM gene involved in each correlation. This resulted in six cell lines with the highest and six cell lines with the lowest expression of the OCM gene for each specific correlation. We used the Student's *t*-test to test whether the NCI-60 cell lines groups with high and low folate gene expression had statistically significantly different average drug response *z*-scores. The resulting *p*-values were FDR adjusted. The effect sizes for the comparison of drug response between the highest and the lowest 10% of the OCM gene-expressing cell lines were estimated using Cohen's *d,* which was computed using the numpy package v. 1.11.3 [37]. In addition to comparing the differences in drug sensitivity between the NCI-60 cell lines with high and

Table 1 One-carbon metabolism genes which were associated with sensitivity to antitumor agents with $|r| > 0.3$ and FDR adjusted $p < 0.05$.

Agent	Targets	Associated gene	Pearson *r*	FDR adjusted *p*-value	Sample size	Spearman	Targeted pathway
Dasatinib	ABL; SRC; Ephrins; PDGFR; KIT	*NNMT*	−0.3408	3.31×10^{-6}	220		ABL signaling
GNF-2	BCR-ABL	*GART*	−0.3351	4.47×10^{-6}	222	*	ABL signaling
GNF-2	BCR-ABL	*TYMS*	−0.3159	1.72×10^{-5}	222	*	ABL signaling
Imatinib	ABL; KIT; PDGFR	*GART*	−0.3124	1.66×10^{-5}	228		ABL signaling
Nilotinib	ABL	*GART*	−0.3125	4.31×10^{-11}	514		ABL signaling
WH-4-023	SRC; LCK	*NNMT*	−0.3154	1.95×10^{-5}	220		ABL signaling
ABT-263	BCL2; BCL-XL; BCL-W	*TYMS*	−0.3121	1.57×10^{-11}	538		Apoptosis regulation
XMD13-2	RIPK1	*GART*	−0.3382	1.24×10^{-14}	595	*	Apoptosis regulation
XMD13-2	RIPK1	*NNMT*	0.3084	2.34×10^{-12}	595	*	Apoptosis regulation
XMD13-2	RIPK1	*TYMS*	−0.3008	8.81×10^{-12}	595		Apoptosis regulation
NPK76-II-72-1	PLK3	*GART*	−0.3157	5.90×10^{-13}	596	*	Cell cycle
NPK76-II-72-1	PLK3	*TYMS*	−0.3267	9.61×10^{-14}	596	*	Cell cycle
PHA-793887	CDK2; CDK7; CDK5	*GART*	−0.3332	3.23×10^{-14}	595	*	Cell cycle
PHA-793887	CDK2; CDK7; CDK5	*NNMT*	0.3025	6.61×10^{-12}	595		Cell cycle
EX-527	SIRT1	*SHMT2*	−0.3222	2.41×10^{-13}	591	*	Chromatin histone acetylation
EX-527	SIRT1	*TYMS*	−0.3328	3.86×10^{-14}	591	*	Chromatin histone acetylation
Tubastatin A	HDAC1; HDAC6; HDAC8	*GART*	−0.3208	2.75×10^{-13}	592	*	Chromatin histone acetylation
VNLG/124	HDAC; RAR	*SHMT2*	−0.3318	4.58×10^{-14}	591	*	Chromatin histone acetylation
Vorinostat	HDAC inhibitor Class I; IIa; IIb; IV	*NNMT*	0.3241	2.14×10^{-12}	538	*	Chromatin histone acetylation
I-BET-762	BRD2; BRD3; BRD4	*SHMT2*	−0.3118	1.44×10^{-12}	592		Chromatin other
UNC1215	L3MBTL3	*GART*	−0.3167	7.74×10^{-13}	585	*	Chromatin other
UNC1215	L3MBTL3	*SHMT2*	−0.3354	3.23×10^{-14}	585	*	Chromatin other
UNC1215	L3MBTL3	*TYMS*	−0.3264	1.44×10^{-13}	585	*	Chromatin other
GSK429286A	ROCK1; ROCK2	*MTR*	−0.3247	1.27×10^{-13}	595	*	Cytoskeleton
GSK429286A	ROCK1; ROCK2	*TYMS*	−0.3566	3.41×10^{-16}	595	*	Cytoskeleton
Y-39983	ROCK	*TYMS*	−0.3150	6.62×10^{-13}	596	*	Cytoskeleton
Methotrexate	Antimetabolite	*GART*	−0.3618	4.37×10^{-15}	536	*	DNA replication
Methotrexate	Antimetabolite	*NNMT*	0.3249	2.06×10^{-12}	536	*	DNA replication
Methotrexate	Antimetabolite	*SHMT2*	−0.4032	1.96×10^{-18}	536	*	DNA replication
Methotrexate	Antimetabolite	*TYMS*	−0.3347	3.71×10^{-13}	536		DNA replication
Erlotinib	EGFR	*BHMT*	−0.3592	2.56×10^{-6}	201		EGFR signaling
Lapatinib	ERBB2; EGFR	*BHMT*	−0.3867	8.85×10^{-8}	218		EGFR signaling
BIX02189	MEK5; ERK5	*SLC46A1*	0.3057	3.62×10^{-12}	596	*	ERK MAPK signaling
TL-2-105	Not defined	*GART*	−0.3054	3.94×10^{-12}	595		ERK MAPK signaling
TAK-715	p38α; p38β	*GART*	−0.3159	5.90×10^{-13}	595		JNK and p38 signaling
TAK-715	p38α; p38β	*SHMT2*	−0.3237	1.44×10^{-13}	595	*	JNK and p38 signaling

(*continued on next page*)

Table 1 (*continued*)

Agent	Targets	Associated gene	Pearson *r*	FDR adjusted *p*-value	Sample size	Spearman	Targeted pathway
VX-702	p38	*GART*	−0.3139	1.41×10^{-11}	534	*	JNK and p38 signaling
VX-702	p38	*SHMT2*	−0.3070	4.25×10^{-11}	534	*	JNK and p38 signaling
FK866	NAMPT	*TYMS*	−0.3590	4.12×10^{-16}	582	*	Metabolism
Genentech Cpd 10	AURKA; AURKB	*MTR*	−0.3004	9.29×10^{-12}	595	*	Mitosis
GSK1070916	AURKA; AURKC	*GART*	−0.3197	6.69×10^{-13}	577	*	Mitosis
GSK1070916	AURKA; AURKC	*MTR*	−0.3302	1.20×10^{-13}	577		Mitosis
GSK1070916	AURKA; AURKC	*TYMS*	−0.3724	5.77×10^{-17}	577	*	Mitosis
VX-680	AURKA; AURKB; AURKC; others	*TYMS*	−0.3386	4.07×10^{-6}	219	*	Mitosis
Cyclopamine	SMO	*SLC46A1*	0.3013	5.57×10^{-5}	217	*	Other
KIN001-135	IKK	*GART*	−0.3061	3.61×10^{-5}	220	*	Other
KIN001-135	IKK	*MTHFD2*	−0.3079	3.18×10^{-5}	220	*	Other
KIN001-236	Angiopoietin-1 receptor	*SHMT2*	−0.3371	1.40×10^{-14}	596	*	Other
KIN001-260	IKKB	*GART*	−0.3014	7.61×10^{-12}	596		Other
KIN001-260	IKKB	*SHMT2*	−0.3360	1.72×10^{-14}	596	*	Other
KIN001-260	IKKB	*SLC46A1*	0.3043	4.54×10^{-12}	596	*	Other
QL-XI-92	DDR1	*GART*	−0.3327	3.23×10^{-14}	595		Other
QL-XI-92	DDR1	*MTR*	−0.3205	2.56×10^{-13}	595	*	Other
QL-XI-92	DDR1	*TYMS*	−0.3272	9.56×10^{-14}	595	*	Other
STF-62247	Autophagy inducer	*GART*	−0.3177	5.10×10^{-13}	592		Other
STF-62247	Autophagy inducer	*SHMT2*	−0.3429	5.43×10^{-15}	592	*	Other
STF-62247	Autophagy inducer	*TYMS*	−0.3022	7.78×10^{-12}	592	*	Other
T0901317	LXR; FXR	*GART*	−0.3259	1.27×10^{-13}	591	*	Other
T0901317	LXR; FXR	*SHMT2*	−0.3444	4.72×10^{-15}	591	*	Other
T0901317	LXR; FXR	*TYMS*	−0.3057	4.45×10^{-12}	591	*	Other
WZ3105	SRC; ROCK2; NTRK2; FLT3; IRAK1; others	*NNMT*	0.3247	1.27×10^{-13}	595	*	Other
XMD14-99	ALK; CDK7; LTK; others	*GART*	−0.3265	1.03×10^{-13}	595	*	Other
XMD14-99	ALK; CDK7; LTK; others	*MTR*	−0.3239	1.44×10^{-13}	595		Other
XMD14-99	ALK; CDK7; LTK; others	*SHMT2*	−0.3794	3.54×10^{-18}	595	*	Other

(*continued on next page*)

Table 1 (*continued*)

Agent	Targets	Associated gene	Pearson *r*	FDR adjusted *p*-value	Sample size	Spearman	Targeted pathway
XMD8-85	ERK5; BET	*SLC46A1*	0.3306	7.69×10^{-6}	218	*	Other
Z-LLNle-CHO	gamma-secretase	*ALDH2*	0.3185	1.45×10^{-5}	222	*	Other
Z-LLNle-CHO	gamma-secretase	*MAT2B*	−0.3341	4.83×10^{-6}	222	*	Other
Zibotentan ZD4054	EDNRA	*GART*	−0.3147	6.69×10^{-13}	596	*	Other
Zibotentan ZD4054	EDNRA	*SHMT2*	−0.3593	2.28×10^{-16}	596	*	Other
Zibotentan ZD4054	EDNRA	*TYMS*	−0.3420	5.43×10^{-15}	596	*	Other
BMS-345541	IKK1; IKK2	*NNMT*	0.3039	5.08×10^{-12}	595		Other; kinases
CEP-701	FLT3; JAK2; NTRK1; NTRK2; NTRK3	*TYMS*	−0.3328	5.16×10^{-13}	536	*	Other; kinases
FMK	RSK	*GART*	−0.3024	8.16×10^{-11}	535	*	Other; kinases
FMK	RSK	*SHMT2*	−0.3054	5.03×10^{-11}	535	*	Other; kinases
QL-XII-61	BMX; BTK	*SLC46A1*	0.3015	3.33×10^{-6}	284	*	Other; kinases
QL-XII-61	BMX; BTK	*TYMS*	−0.3015	3.33×10^{-6}	284		Other; kinases
TL-1-85	TAK	*SLC46A1*	0.3122	1.08×10^{-12}	596	*	Other; kinases
XMD15-27	CAMK2	*GART*	−0.3083	2.34×10^{-12}	595		Other; kinases
XMD15-27	CAMK2	*MTR*	−0.3255	1.20×10^{-13}	595		Other; kinases
XMD15-27	CAMK2	*SHMT2*	−0.3567	3.41×10^{-16}	595	*	Other; kinases
XMD15-27	CAMK2	*TYMS*	−0.3435	4.72×10^{-15}	595	*	Other; kinases
BX-912	PDK1 (PDPK1)	*MTR*	−0.3067	3.18×10^{-12}	595		PI3K/MTOR signaling
BX-912	PDK1 (PDPK1)	*TYMS*	−0.3625	1.39×10^{-16}	595	*	PI3K/MTOR signaling
AV-951	VEGFR1; VEGFR2; VEGFR3	*GART*	−0.3207	2.50×10^{-13}	595	*	RTK signaling
AV-951	VEGFR1; VEGFR2; VEGFR3	*TYMS*	−0.3471	2.60×10^{-15}	595	*	RTK signaling
Crizotinib	MET; ALK; ROS1	*MAT2B*	−0.3448	1.63×10^{-6}	227	*	RTK signaling
Crizotinib	MET; ALK; ROS1	*MTR*	−0.3265	6.51×10^{-6}	227	*	RTK signaling
Crizotinib	MET; ALK; ROS1	*SHMT2*	−0.3298	5.14×10^{-6}	227	*	RTK signaling
Crizotinib	MET; ALK; ROS1	*SLC46A1*	0.3188	1.14×10^{-5}	227	*	RTK signaling
OSI-930	KIT	*GART*	−0.3038	5.08×10^{-12}	595		RTK signaling
Sunitinib	PDGFR; KIT; VEGFR; FLT3; RET; CSF1R	*MAT2B*	−0.3061	3.29×10^{-5}	222	*	RTK signaling
Sunitinib	PDGFR; KIT; VEGFR; FLT3; RET; CSF1R	*SHMT2*	−0.3170	1.61×10^{-5}	222	*	RTK signaling
Sunitinib	PDGFR; KIT; VEGFR; FLT3; RET; CSF1R	*SLC46A1*	0.3197	1.35×10^{-5}	222	*	RTK signaling
CHIR-99021	GSK3A; GSK3B	*SLC46A1*	0.3124	2.41×10^{-13}	630		WNT signaling

Pearson *r*: Pearson correlation coefficient; **Spearman**: an asterisk (*) indicates correlations which also satisfied $\rho > 0.3$ and FDR adjusted $p < 0.05$ for Spearman correlation.
Information about molecular drug targets and target pathways for each agent listed in Table 1 was obtained from the GDSC data download site [25]. Additional information about ABL signaling inhibitors was included based on data from Supplementary Table S1F of Iorio et al. [27]. An expanded list of potential drug targets for each agent is provided in Supplementary Table 1.

low OCM gene expression, we also used Pearson correlation analysis to evaluate the associations between OCM gene expression and drug response for these 6 drug-gene pairs in the entire NCI-60 dataset.

Analysis of correlation of expression among OCM genes, and of OCM gene expression with regulatory genes and genes involved in drug target pathways

We evaluated possible joint effects of OCM genes on drug sensitivity and a possible co-regulation among OCM genes by examining Pearson correlation among expression levels of the 34 OCM genes. In addition, we examined possible molecular mechanisms that could influence the associations of OCM gene expression with drug response by analyzing Pearson correlations between OCM gene expression and expression levels of molecular targets and components of target pathways for the 51 agents listed in Table 1. These gene targets and components of drug target pathways are listed in Supplementary Table 1. Information about gene targets and classification of target pathways for each agent was obtained from the GDSC data download site [25] (Table 1), with additional information about gene targets and components of target pathways collected from the DrugBank [38,39], Cancer Therapeutics Response Portal v. 2 at the Broad Institute [40,41], Drug SIGnatures DataBase (DSigDB) v. 1.0, [42,43], PharmGKB [44,45], PubChem [46], online resources from drug suppliers and manufacturers including Selleck Chemicals [47], Tocris [48], Aphios [49], and AstraZeneca [50], and original publications [51–66]. The full list of gene targets and target pathway components, which also included several OCM genes, is listed in Supplementary Table 1. Gene expression information was available from the CCLE dataset [22] for 532 of these additional target pathway components that were not already a part of the list of 34 candidate OCM genes. To examine possible associations of *c*-Myc and HIF-1 (hypoxia-inducible factor 1) transcriptional levels and OCM gene expression, we also analyzed Pearson correlations of expression levels of the *MYC* and *HIF1A* genes with baseline expression of the 34 OCM genes in untreated cells. FDR adjustment of the *p*-values derived from the analysis of Pearson correlations of OCM gene expression with other OCM genes and with target pathway components from the top 51 significantly associated agents listed in Table 1 and in Supplementary Table 1 accounted for the comparisons involving 567 genes that included *HIF1A,* 34 OCM genes, and 532 additional genes (including *MYC*) that had been reported to be involved in drug target pathways. Because analysis of correlations among $\log_2$-transformed RMA-normalized gene expression levels did not involve drug response measures, it was not restricted to the 635 cell lines with matching GDSC drug response data. This analysis was based on a broader dataset of 1036 cell lines with Affymetrix Human Genome U133 Plus 2.0 microarray data, available from CCLE [22].

Information about chromosomal assignment and genome locations of candidate genes was obtained from GeneCards [67,68].

Regression analysis of association of cell line response to kinase inhibitors with OCM gene expression and mutational status of molecular drug targets

Among the top agents associated with OCM gene expression (Table 1; Supplementary Table 1), sensitivity and resistance to three kinase inhibitors, erlotinib, lapatinib, and crizotinib, have been previously associated with a number of well-defined genomic alterations and DNA and protein sequence changes in their molecular target genes. We examined which of the genomic alterations known to affect sensitivity or resistance were present in the cell lines with available response data to these three agents. Gene amplification and fusion status was determined using the data from the CCLE web resource of the Broad Institute [22]. Gene level copy number data had been generated by the CCLE Consortium using Affymetrix 6.0 SNP arrays, with segmentation of normalized $\log_2$ ratios of the copy number estimates performed using the circular binary segmentation algorithm [22]. We defined gene amplification status as ≥ 5 copies of a gene (i.e., $\log_2$ of normalized values for a diploid genome ≥ 1.322). Data on DNA sequence variants resulting in protein sequence changes were obtained from the whole exome sequencing information downloaded from the GDSC online resource [25].

Among the three agents, the data for more than one cell line with genomic alterations affecting sensitivity were available only for crizotinib response. We examined four folate genes (*MAT2B, MTR, SLC46A1*, and *SHMT2*), expression of which was significantly correlated with log(IC50) of crizotinib (Table 1). We used multiple regression to investigate whether the expression of each of these genes remained a significant predictor of cancer cell line response to crizotinib after accounting for the presence of genomic alterations with known effects on crizotinib sensitivity and resistance. Expression of each of the four OCM genes, *MAT2B, MTR, SLC46A1*, and *SHMT2,* was analyzed separately, and their regression *p*-values were adjusted for multiple testing using the FDR approach. We used expression of each folate gene, the presence of a genomic alteration with reported effects on crizotinib sensitivity, and the presence of a genomic alteration with reported effects on crizotinib resistance as dependent variables in linear regression modeling, whereas log(IC50) of crizotinib was used as the outcome variable.

Results

Association of OCM gene expression with drug response

We investigated whether cancer drug response was associated with one-carbon metabolism processes by analyzing Pearson correlation between baseline OCM gene expression and log(IC50) values of cancer cell line response to drug treatment. Table 1 provides the list of genes which were associated with sensitivity to antitumor agents (Pearson $|r| \geq 0.3$, FDR-adjusted $p < 0.05$). Ten genes, *GART, TYMS, SHMT2, MTR, ALDH2, BHMT, MAT2B, MTHFD2, NNMT*, and *SLC46A1,* satisfied these criteria. Fig. 1 illustrates several top correlations from Table 1. Pretreatment expression levels of 8 of

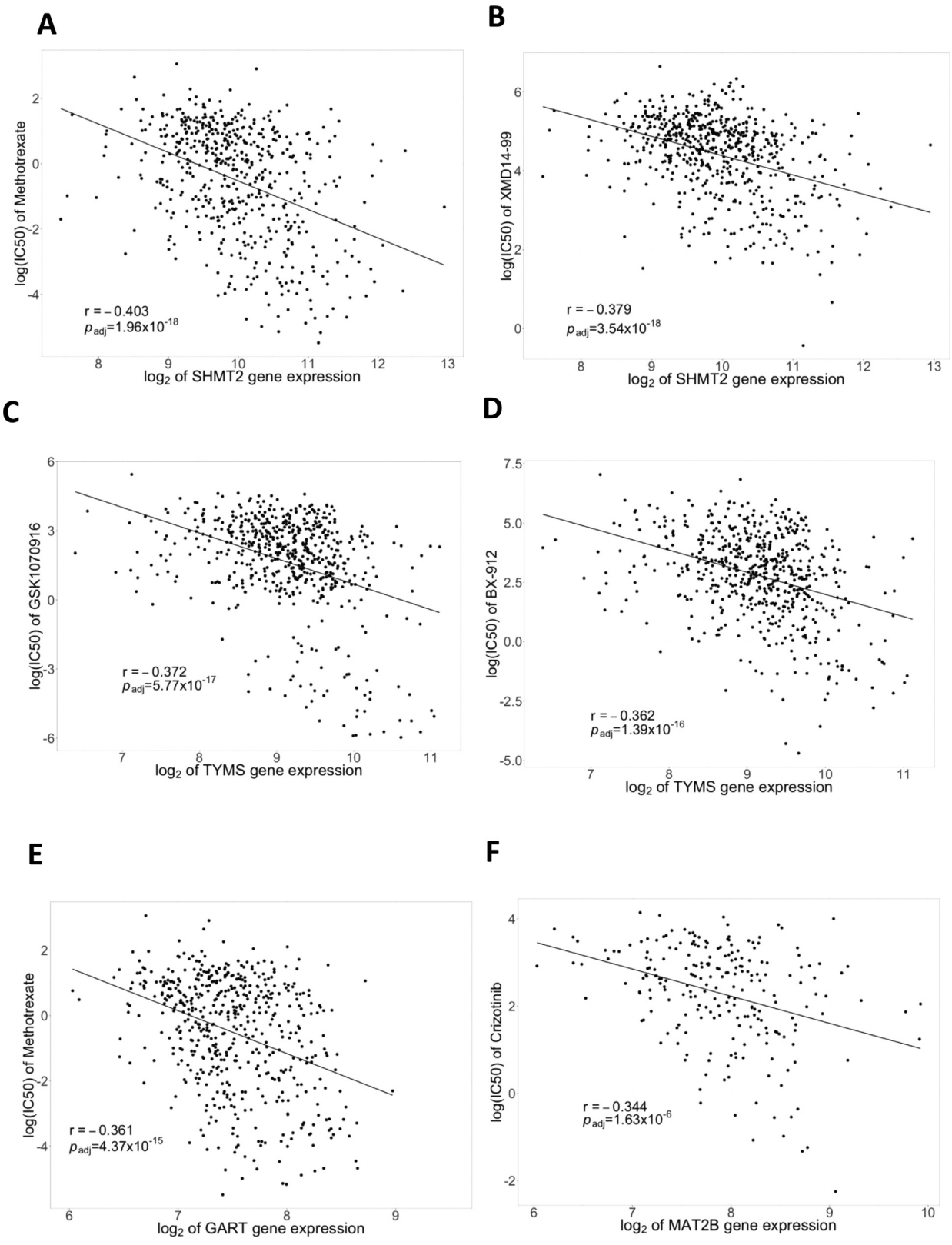

Fig. 1 Scatterplots of selected top correlations of gene expression with drug response from Table 1 satisfying $|r| \geq 0.3$ and FDR adjusted $p < 0.05$. Horizontal axis represents $\log_2$ of cell line gene expression from the CCLE microarray expression dataset; vertical axis represents log(IC50) of drug response. **(A)** *SHMT2* vs methotrexate; **(B)** *SHMT2* vs XMD14-99; **(C)** *TYMS* vs GSK1070916; **(D)** *TYMS* vs BX-912; **(D)** *GART* vs methotrexate; **(E)** *MAT2B* vs crizotinib.

the 10 genes listed in Table 1, *GART, TYMS, SHMT2, MTR, BHMT, MAT2B, NNMT*, and *SLC46A1*, were each correlated with response to multiple drugs. Even though these correlations were highly significant (FDR adjusted $p < 0.0001$ for all associations in Table 1), they were relatively weak ($|r| \leq 0.403$).

Methotrexate, an antifolate drug targeting DHFR (dihydrofolate reductase), had the strongest association ($r = -0.403$ for correlation with *SHMT2* expression; Fig. 1A). This result was partially validated in the NCI-60 dataset, in which sensitivity to methotrexate was also strongly associated with *SHMT2* expression when using Pearson correlation in all available cell lines, which was statistically significant ($r = -0.456$, FDR adjusted $p = 0.0017$). However, while there was a considerable difference in sensitivity between the six highest and the six lowest *SHMT2*-expressing cell lines, this difference was not statistically significant (Cohen's $d = -1.0752$, FDR adjusted $p = 0.2104$; Supplementary Table 2).

In the GDSC–CCLE dataset, sensitivity to methotrexate was also associated ($r = -0.362$, -0.335, and 0.325) with elevated expression of *GART* (the phosphoribosylglycinamide formyltransferase gene), *TYMS* (encoding thymidylate synthase), and *NNMT* (the nicotinamide N-methyltransferase gene), respectively (Table 1 and Fig. 1E). The log(IC50) values of methotrexate also showed a weak, but statistically significant ($r = -0.245$, $p = 1.96 \times 10^{-7}$) correlation with expression of the *DHFR* gene, which encodes the target of this agent. These results suggest that cell sensitivity to this antifolate agent may be influenced by expression of several components of the OCM pathway. While association of *GART* expression with sensitivity to methotrexate was not significant in the NCI-60 panel (Supplementary Table 2), the observed correlation of increased expression of several OCM genes with methotrexate sensitivity in the GDSC-CCLE dataset was consistent with an earlier qualitative analysis of a subset of GDSC tumor cell lines and of patient samples, which had found a statistically significant association of elevated baseline *SHMT2, GART, TYMS*, and *DHFR* expression in tumor cell lines with methotrexate sensitivity and of *SHMT2* expression in acute lymphoblastic leukemia (ALL) patients with response to methotrexate treatment [12]. This association of increased baseline expression of OCM genes with drug sensitivity is notable and is in contrast to overexpression of *DHFR* and *TYMS* that tumors develop in response to treatment, which serves as a mechanism of acquired resistance to anti-folate drugs methotrexate, pemetrexed, and 5-fluorouracil [20,69].

Increased expression of *SLC46A1,* encoding a proton-coupled folate transporter, was weakly associated with higher levels of resistance to 9 agents including BIX02189, CHIR-99021, crizotinib, cyclopamine, KIN001-260, QL-XII-61, sunitinib, TL-1-85, and XMD8-85 (r between 0.301 and 0.330; Table 1). The product of this gene participates in the transport of folates and antifolates. Hypermethylation of the *SLC46A1* promoter was previously found to be associated with HeLa cell resistance to methotrexate [70]. Agents associated with *SLC46A1* expression belong to classes other than antifolate drugs (Table 1). It could be possible that an association of elevated expression of this gene with increased resistance to several agents could be an indirect consequence of an increased folate transport to cancer cells.

While the overwhelming majority of the 34 genes examined in our study had unimodal patterns of expression, the distribution of expression levels of *NNMT, ALDH2*, and *CBS* in the GDSC-CCLE dataset was bimodal. Fig. 2 shows the bimodal pattern of *NNMT* expression and unimodal expression of *SLC46A1, GART, TYMS, SHMT2*, and *MTR*. Fig. 2C indicates that many cell lines had no or very low levels of *NNMT* expression, whereas the expression of this gene in other cell lines was at considerable levels (Fig. 2C). The bimodality of *NNMT* gene expression and of protein expression and activity of NNMT in human liver has been well established, and multiple studies have suggested that differences in *NNMT* expression among patients affect metabolic rates, therapeutic response to treatment, and drug toxicity [71–73]. Our analysis using CCLE legacy online portal tools [23] showed that while average *NNMT* expression values differed among cancer categories, with very low or no expression of that gene observed in most leukemia and multiple myeloma cell lines, the majority of other cancer categories included both high and low *NNMT* expressing individual cell lines (data not shown).

Nicotinamide N-methyltransferase, the product of *NNMT*, is an important drug target. It is involved in the conversion of nicotinamide to 1-methylnicotinamide that uses *S*-adenosylmethionine as a methyl donor, in methylation of pyridines and their structural analogs, and in biotransformation of drug agents [74–77]. NNMT plays important roles in oncogenesis and cancer progression and is overexpressed in a variety of cancers, resulting in aberrant methylation of cancer-related proteins, and its phosphorylation is inhibited by an ALK inhibitor, RO5424802 [75,78,79]. Downregulation of NNMT was observed to induce apoptosis in breast cancer cell lines [72]. In our study, higher *NNMT* gene expression levels were associated with sensitivity to dasatinib ($r = -0.340$) and to an Lck/Src inhibitor, WH-4-023 ($r = -0.315$). In contrast, they were also weakly (r between 0.302 and 0.325) associated with chemoresistance to six additional agents from several classes (Table 1). Among these agents, association of *NNMT* expression with chemoresistance to methotrexate, vorinostat, WZ3105, and XMD13-2 also satisfied the criteria of $|\rho| > 0.3$ and FDR adjusted $p < 0.05$ in Spearman correlation analysis (Table 1), demonstrating the robustness of this association even though the *NNMT* gene expression was bimodally distributed. Some studies have suggested that genes with bimodal transcriptional patterns may serve as diagnostic and prognostic cancer biomarkers [80,81]. Based on the biological importance of NNMT in cancer [72,75,78,79], bimodality of its gene expression, and the statistical association of *NNMT* gene expression with cellular response to multiple drugs, this gene and its product may be of potential clinical interest in cancer treatment.

Association of elevated baseline *NNMT* expression with dasatinib sensitivity was further validated in the NCI-60 dataset, in which we observed a statistically significant difference in drug sensitivity values between the cell lines with the highest and the lowest *NNMT* expression, with a very large effect size (Cohen's $d = -2.179$, FDR adjusted $p = 0.0219$; Supplementary Table 2). Pearson correlation analysis of the entire NCI-60 dataset also showed a statistically significant correlation between *NNMT* expression and sensitivity to dasatinib ($r = -0.364$, FDR adjusted $p = 0.0156$; Supplementary Table

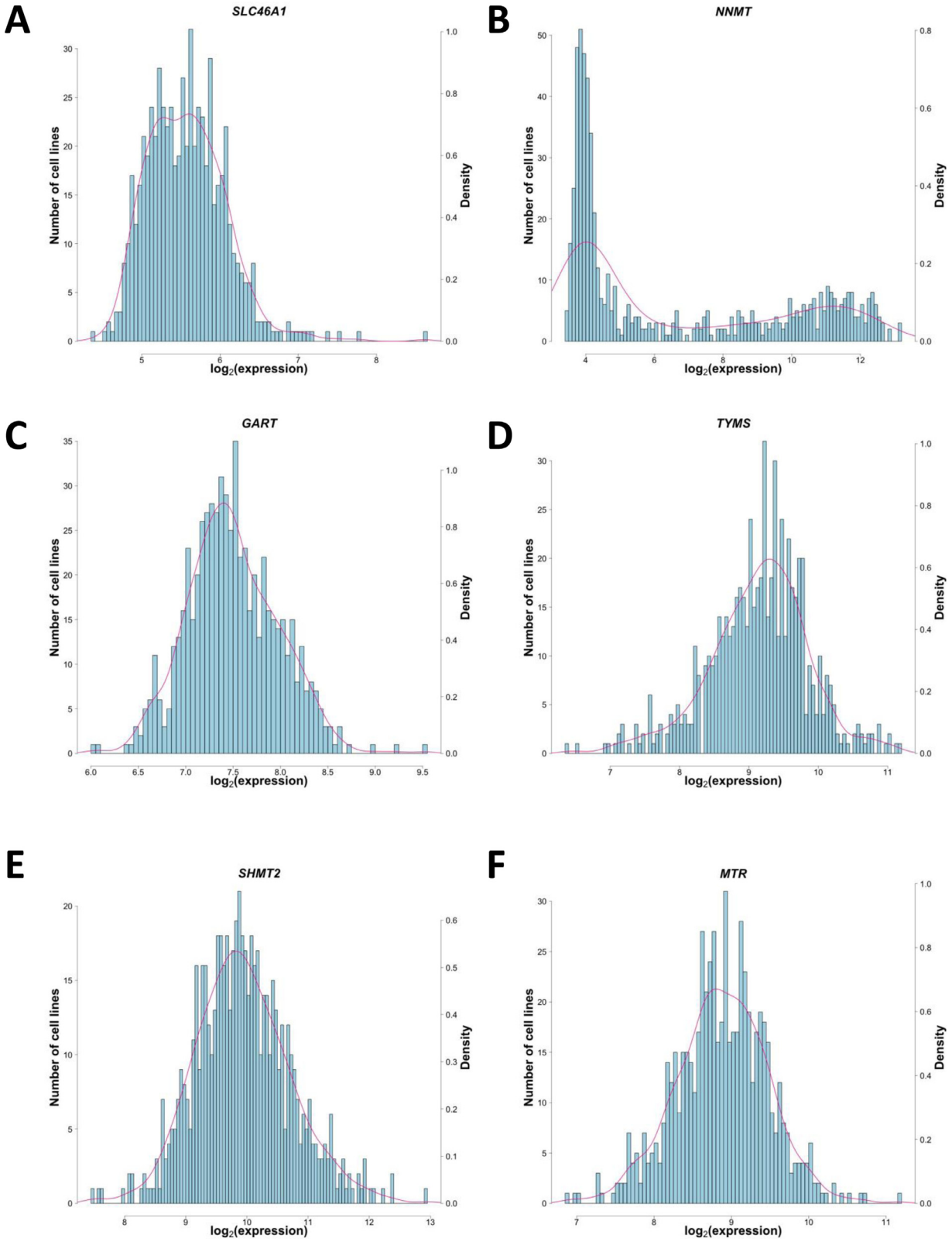

Fig. 2 Histograms and density functions showing the distributions of expression values of selected folate metabolism-related genes among 635 cell lines with available CCLE gene expression data that had matching GDSC drug response data. **(A)** *SLC46A1*; **(B)** *NNMT*; **(C)** *GART*; **(D)** *TYMS*; **(E)** *SHMT2*; **(F)** *MTR*. Horizontal scale represents $\log_2$-transformed gene expression values. The left vertical scale represents cell line counts, whereas the right vertical scale represents density values.

2). Our results were in agreement with a prior report by Huang et al. [82] who found an association of *NNMT* expression with dasatinib sensitivity; in contrast, Chen et al. [83] reported that *NNMT* expression was a biomarker of resistance to dasatinib. In agreement with our findings of divergent directions of association with sensitivity to treatment, *NNMT* expression has been reported to be associated with resistance to radiation and to several cancer drugs, but with sensitivity to a sulphonamide cryptopleurine analogue [74].

Previous reports had suggested that *BHMT*, which encodes betaine-homocysteine methyltransferase, has a protective role against cancer, and its deletion in mouse models promoted fatty liver disease and hepatocellular carcinoma [84,85]. Higher levels of *BHMT* expression were associated with sensitivity to kinase inhibitors erlotinib and lapatinib ($r=-0.359$ and -0.387, respectively; Table 1). Our examination of these associations in the NCI-60 dataset (Supplementary Table 2) showed a partial validation of the *BHMT*-erlotinib association, which was significant when using Pearson correlation analysis in the full NCI-60 dataset ($r=-0.349$, FDR adjusted $p=0.0156$), but the difference in erlotinib sensitivity between the highest and the lowest *BHMT*-expressing cell lines was not statistically significant. No significant association of *BHMT* expression with log(GI50) of lapatinib was observed in the NCI-60 dataset (Supplementary Table 2).

The increased expression of *MAT2B*, which encodes the beta subunit of the *L*-methionine *S*-adenosyltransferase II that participates in the biosynthesis of *S*-adenosylmethionine (SAM) and contributes to growth of colon and liver cancer cells [78], was associated in the GDSC-CCLE dataset with sensitivity to an ALK inhibitor crizotinib ($r=-0.345$), a kinase inhibitor sunitinib ($r=-0.306$), and a gamma-secretase inhibitor Z-LLNle-CHO ($r=-0.334$; Table 1); however, in the NCI-60 dataset the association of *MAT2B* expression with crizotinib sensitivity was not significant (Supplementary Table 2). In the GDSC–CCLE dataset, higher expression levels of *ALDH2*, the product of which affects folate levels *in vivo* [86], were associated with resistance to Z-LLNle-CHO ($r=0.319$). Elevated expression of *MTHFD2,* the product which is involved in the mitochondrial OCM pathway, was weakly ($r=-0.308$) associated with sensitivity to a small molecule IKKε inhibitor, KIN001-135. This gene is overexpressed in many cancer categories and is required for survival of malignant cells [87]. Elevated expression levels of *MTHFD2* had been associated with shorter survival rate of patients with colorectal cancer and lung adenocarcinoma [88].

Increased expression of *GART, TYMS, SHMT2*, and *MTR* were associated with sensitivity to multiple agents (Table 1; Figs. 1 and 3). Expression levels of *GART* and *TYMS*, both of which encode products involved in nucleotide synthesis, were negatively associated with log(IC50) of 24 and 21 drugs, respectively (r between -0.301 and -0.362 for *GART* and -0.301 and -0.372 for *TYMS*). Overexpression of *TYMS* and *GART* had been associated with a late relapse of childhood acute lymphoblastic leukemia [89]. Additionally, elevated TYMS gene and protein expression had been correlated with poorer overall survival of colorectal patients, and some studies also reported an association of a tandem repeat polymorphism in the *TYMS* gene promoter with survival of patients with colorectal cancer [69,90]. Similarly, GART protein overexpression had been associated with poorer overall survival of glioma patients and with progression and overall survival of hepatocellular carcinoma [91,92].

Table 2 Pearson correlation results among expression levels of one-carbon metabolism genes which satisfied $|r| > 0.3$ and FDR adjusted $p < 0.05$.

OCM Gene 1	OCM Gene 2	*r*	FDR adjusted *p*
DHFR	*TYMS*	0.5753	4.48×10^{-89}
ATIC	*GART*	0.4270	1.02×10^{-44}
AHCY	*ATIC*	0.4211	2.21×10^{-43}
GART	*SLC19A1*	0.4161	3.03×10^{-42}
ATIC	*MTHFD1*	0.3957	6.36×10^{-38}
ATIC	*SLC19A1*	0.3894	1.20×10^{-36}
MTHFD1	*TYMS*	0.3883	1.94×10^{-36}
CBS	*PHGDH*	0.3860	5.63×10^{-36}
GART	*NNMT*	−0.3549	3.61×10^{-30}
CTH	*SHMT2*	0.3547	3.87×10^{-30}
DHFR	*SHMT1*	0.3528	8.30×10^{-30}
DHFR	*MTHFD1*	0.3481	5.49×10^{-29}
GART	*MTHFD2L*	0.3421	5.60×10^{-28}
DHFR	*GART*	0.3420	5.78×10^{-28}
SLC19A1	*TYMS*	0.3407	9.50×10^{-28}
GART	*MTHFD1*	0.3392	1.67×10^{-27}
GART	*TYMS*	0.3338	1.30×10^{-26}
GART	*MAT2A*	0.3316	2.95×10^{-26}
AHCY	*SLC19A1*	0.3310	3.59×10^{-26}
DHFR	*MAT2A*	0.3299	5.44×10^{-26}
PEMT	*SHMT1*	0.3235	5.50×10^{-25}
DHFR	*SLC19A1*	0.3191	2.77×10^{-24}
MAT2A	*SLC19A1*	0.3181	3.96×10^{-24}
AHCY	*MTHFD1*	0.3035	5.92×10^{-22}
MTHFD1	*SHMT1*	0.3031	6.54×10^{-22}
SHMT1	*TYMS*	0.3026	7.74×10^{-22}
MTHFD2	*SHMT2*	0.3026	7.74×10^{-22}

r: Pearson correlation coefficient.
Correlation results listed in the table are based on 1036 CCLE cell lines with available expression microarray information.

Expression of *SHMT2,* which encodes mitochondrial serine hydroxymethyl transferase 2 and is involved in glycine biosynthesis [7], was correlated with response to 17 agents (r between -0.305 and -0.403). An earlier study had shown an association of increased *SHMT2* gene expression with shorter overall survival rates of patients with colorectal cancer and lung adenocarcinoma [88]. Expression of the methionine synthase gene *MTR*, which affects cellular methylation processes by participating in remethylation of homocysteine, was associated with 8 agents (r between -0.300 and -0.330). An earlier meta-analysis of case-control patient data had suggested an association of the A2756G polymorphism in that gene with susceptibility to acute lymphoblastic leukemia and colorectal cancer [93].

Expression levels of multiple one-carbon metabolism genes were correlated

Table 2 shows the most prominent statistically significant correlations among expression levels of OCM genes which satisfied Pearson $|r| > 0.3$ and FDR adjusted $p < 0.05$. Expression

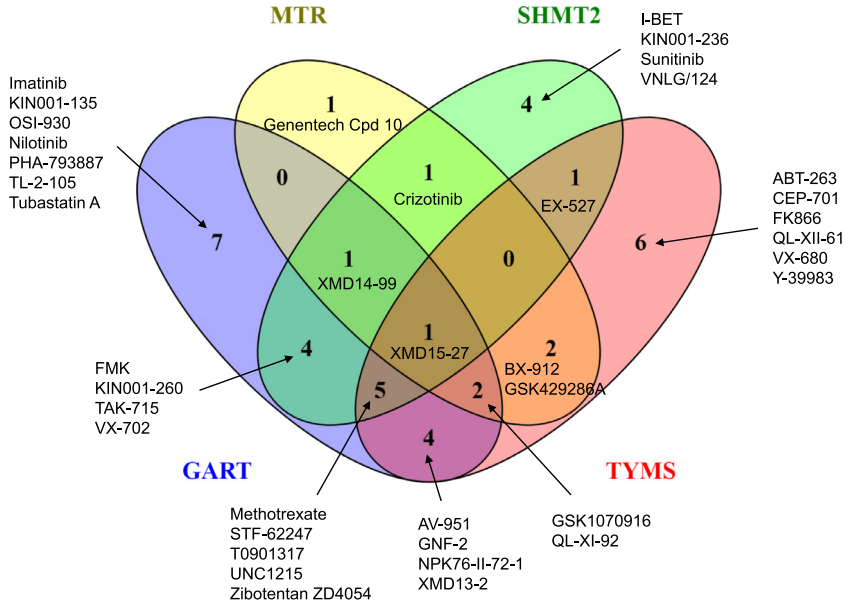

Fig. 3 Antitumor agents associated with *GART, MTR, SHMT2*, and *TYMS* gene expression from Table 1, satisfying Pearson $|r| > 0.3$ and FDR adjusted $p < 0.05$.

levels were positively correlated among many OCM genes, including 26 out of the 27 correlations listed in Table 2, which is consistent with previously reported associations in ALL patients [94]. This suggests a possibility that expression of some of these genes in cancer cells may be co-regulated. The strongest correlation was observed between expression levels of *TYMS* and *DHFR* ($r = 0.575$, FDR adjusted $p = 4.48 \times 10^{-89}$). Previously, we observed that both genes had similar patterns of expression response to several anticancer drugs in the NCI-60 cell lines [21]. Products of these genes catalyze related reactions in the OCM pathway. TYMS catalyzes the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF) to dihydrofolate (DHF) during the conversion of deoxyuridine monophosphate into deoxythymidine monophosphate in the process of thymidylate biosynthesis, whereas the DHFR catalyzes the reduction of DHF to tetrahydrofolate (THF), and also the conversion of dietary folate to DHF and then to THF [8,85,95]. The *TYMS* and *DHFR* genes are located in separate genome regions on different chromosomes (18p11.32 and 5q14.1, respectively), suggesting that their co-regulation is likely related to their functional activity rather than to their chromosomal co-localization.

Other strong positive associations included correlations between *ATIC* and *GART* ($r = 0.427$), both of which are involved in purine biosynthesis [96], *ATIC* and *AHCY* ($r = 0.421$), and *GART* and *SLC19A1* ($r = 0.416$). Twenty-two additional correlations including the above 6 genes *DHFR, TYMS, AHCY, ATIC, GART,* and *SLC19A1* and 8 additional OCM genes (*CBS, CTH, MAT2A, MTHFD1, MTHFD2L, PEMT, PHGDH*, and *SHMT1*) had weaker but also positive associations (r between 0.30 and 0.40; Table 2).

NNMT expression, and to a lesser extent that of *TCN2, ALDH2, SLC46A1, FOLR1,* and *FOLR3,* was very weakly negatively correlated with multiple other OCM genes (Supplementary Table 3); however only the negative correlation between *NNMT* with *GART* expression exceeded the threshold or $r < 0.3$ ($r = -0.355$, $p = 3.61 \times 10^{-30}$; Table 2).

Expression of one-carbon metabolism genes was strongly correlated with transcriptional levels of multiple components of drug target pathways

Expression of many genes encoding direct drug targets or target pathway components of the drug agents listed in Table 1 was strongly correlated with expression levels of multiple OCM genes. The complete list of Pearson correlation results between OCM genes and components of target pathways for each agent is provided in Supplementary Table 4. The strongest significant correlations (Pearson $|r| > 0.45$, FDR adjusted $p < 0.05$) are shown in Table 3. *NNMT* had the strongest associations with molecular drug targets, with 21 associations that had $|r|$ between 0.453 and 0.693 (Table 3), and the total of 90 associations satisfying a weaker threshold of $|r| > 0.3$. *DHFR* and *TYMS* also had multiple significant correlations with $|r| > 0.45$. Other genes that were strongly associated with expression of molecular targets included *MTHFD1, MTHFD2L, GART,* and *SHMT1* ($|r| > 0.45$; Table 3). In addition, expression of these and other OCM-related genes including *AHCY, ATIC, BHMT, FTCD, MAT2A, MAT2B, MTHFR, MTHFS, MTR, MTRR, PEMT, SHMT2, ALDH2*, and folate receptors and transporters *FOLR1, FOLR2, SLC19A1,* and *SLC46A1* was correlated with expression of multiple target pathway components using a weaker threshold of $|r| > 0.3$ (Supplementary Table 4). Interestingly, 408 out of 516 significant correlations between OCM and target gene expression in Supplementary Table 4 satisfying $|r| > 0.3$ were positive, including 43 out of 45 strongest associations with $|r| > 0.45$ (Table 3). Strong positive correlations of OCM gene expression with molecular drug targets may provide a possible explanation for associations of OCM genes with drug sensitivity (Table 1; Supplementary Table 1).

Multiple strong positive correlations were observed between expression of OCM genes and genes encoding

Table 3 Strongest Pearson correlations between expression levels of genes encoding drug target pathway components and one-carbon metabolism genes.

OCM gene	Target pathway component	*r*	FDR adjusted *p*
DHFR	*BRCA1*	0.6085	2.89×10^{-102}
DHFR	PLK4	0.5750	5.51×10^{-89}
DHFR	*GMNN*	0.5539	3.62×10^{-81}
DHFR	*BARD1*	0.5320	3.62×10^{-73}
DHFR	*CHEK1*	0.5249	1.95×10^{-71}
DHFR	*CDK1*	0.5170	6.16×10^{-69}
DHFR	*ATAD5*	0.4983	2.94×10^{-63}
DHFR	*AURKB*	0.4873	4.42×10^{-60}
GART	*CHEK1*	0.5040	6.19×10^{-65}
MTHFD1	*APEX1*	0.5362	4.07×10^{-75}
MTHFD1	*CHEK1*	0.4640	9.16×10^{-54}
MTHFD1	*TDP1*	0.4635	1.20×10^{-53}
MTHFD1	*PLK4*	0.4600	9.79×10^{-53}
MTHFD2L	*PPAT*	0.4789	1.00×10^{-57}
NNMT	*AXL*	0.6933	5.49×10^{-145}
NNMT	*IL6*	0.6330	4.16×10^{-113}
NNMT	*RRAS*	0.6182	1.97×10^{-106}
NNMT	*MYLK*	0.6078	4.44×10^{-102}
NNMT	*FGF2*	0.5962	3.18×10^{-97}
NNMT	*VEGFC*	0.5826	8.15×10^{-92}
NNMT	*NRP1*	0.5826	8.15×0^{-92}
NNMT	*ITGB5*	0.5507	4.35×10^{-80}
NNMT	*PLK2*	0.5449	4.44×10^{-78}
NNMT	*NUAK1*	0.5290	9.75×10^{-73}
NNMT	*ITGAV*	0.5077	4.80×10^{-66}
NNMT	*SYK*	−0.4908	4.50×10^{-61}
NNMT	*SHC1*	0.4796	6.37×10^{-58}
NNMT	*LATS2*	0.4761	5.89×10^{-57}
NNMT	*ABL2*	0.4718	8.18×10^{-56}
NNMT	*EPHA2*	0.4703	2.01×10^{-55}
NNMT	*EGFR*	0.4672	1.32×10^{-54}
NNMT	*ACVR1*	0.4634	1.27×10^{-53}
NNMT	*MDM4*	−0.4622	2.67×10^{-53}
NNMT	*MRAS*	0.4559	1.06×10^{-51}
NNMT	*NOTCH2*	0.4525	7.93×10^{-51}
SHMT1	*AURKB*	0.4570	5.77×10^{-52}
TYMS	*GMNN*	0.5782	3.93×10^{-90}
TYMS	*PLK4*	0.5532	5.92×10^{-81}
TYMS	*AURKB*	0.5275	2.88×10^{-72}
TYMS	*CHEK1*	0.5172	5.47×10^{-69}
TYMS	*ATAD5*	0.4952	2.44×10^{-62}
TYMS	*BRCA1*	0.4848	2.23×10^{-59}
TYMS	*BARD1*	0.4755	8.42×10^{-57}
TYMS	*CDK1*	0.4724	5.84×10^{-56}
TYMS	*CDK2*	0.4705	1.84×10^{-55}

Listed are the strongest correlation results (Pearson $|r| > 0.45$ and FDR adjusted $p < 0.05$) of OCM genes with genes encoding drug targets and target pathway components for agents listed in Table 1 and Supplementary Table 1. The results provided in the table are based on 1036 CCLE cell lines with available gene expression microarray data. Supplementary Table 4 provides the complete set of correlation results between the OCM genes and drug target pathway components for which microarray transcriptional measures were available from CCLE.

r: Pearson correlation coefficient.

kinases, e.g. *ABL2, EGFR, CDK1, CDK2, AURKB, CHEK1,* ligands, e.g. *VEGFC,* and activators, e.g. *FGF2* (Table 3), which may suggest a potential explanation for associations between OCM gene expression and cancer cell line sensitivity to a number of kinase inhibitors (Table 1; Fig. 3). For example, expression of *ABL2*, an Abelson family kinase, was significantly positively correlated with *NNMT* expression ($r = 0.472$; $p = 8.18 \times 10^{-56}$; Table 3). Its product, ABL2, is a target of several kinase inhibitors including imatinib, nilotinib, and dasatinib [97]. Sensitivity to those agents was associated with higher expression levels of OCM genes, including an association of *NNMT* with dasatinib sensitivity ($r = -0.340$, $p = 3.31 \times 10^{-6}$; Table 1). Since *ABL2* expression increases with tumor progression in solid tumors [97], it may be of potential interest to investigate whether expression levels of OCM genes may also change during cancer progression.

Expression of several members of the *ERBB* family of receptor tyrosine kinase genes was significantly correlated with OCM genes, including both positive and negative associations. The strongest correlation was a positive association between *NNMT* expression and expression of the epidermal growth factor receptor gene, *EGFR* ($r = -0.467$, $p = 1.32 \times 10^{-54}$; Table 3). In contrast, *EGFR* expression had a weaker negative correlation with expression of *TYMS, MTR, GART,* and *DHFR* (*r* between −0.373 and −0.325; $p \leq 2.82 \times 10^{-25}$; Supplementary Table 4). Two other *ERBB* family members, *ERBB2* and *ERBB3*, were significantly ($p \leq 3.39 \times 10^{-22}$) associated with expression of the folate transporter *SLC46A1* gene ($r = 0.356$ for *ERBB2* and 0.382 for *ERBB3*), folate receptor *FOLR1* gene ($r = 0.310$ for *ERBB2*), and *MTR* ($r = -0.305$ for *ERBB3*). Interestingly, only *BHMT* showed significant moderate correlations with erlotinib and lapatinib which target *EGFR* and *ERBB2* [98]; however, *BHMT* expression did not correlate with expression levels of either *EGFR, ERBB2,* or *ERBB3* (Supplementary Table 4). Therefore, molecular mechanisms of association between *BHMT* expression and log(IC50) of erlotinib and lapatinib require further investigation.

Increased expression of several OCM genes was significantly ($p \leq 5.27 \times 10^{-27}$) positively correlated with transcriptional levels of aurora kinase A and B genes. Among them, *TYMS, DHFR* and *SHMT1* were strongly correlated with elevated expression of *AURKB* ($r = 0.527$, 0.487, and 0.457, respectively; Table 3), whereas *MTHFD1* expression was to a lesser extent correlated with both *AURKB* and *AURKA* ($r = 0.404$ and 0.336, respectively; Supplementary Table 4). This association may explain modest correlations of higher expression levels of *TYMS, MTR,* and *GART* with sensitivity to aurora kinase inhibitors VX-680, GSK1070916, and Genentech Cpd 10 (*r* between −0.300 and −0.372; Table 1). Because Aurora kinases are commonly overexpressed in cancer [99], positive correlations between OCM gene expression and *AURKA* or *AURKB* expression could potentially also arise due to progression in tumorigenesis rather than through direct co-regulation of these pathways.

We observed multiple significant correlations with targets of epigenetic drugs, which were relatively weak ($0.30 < |r| < 0.36$; Supplementary Table 4). Expression of *BRD2,* one of the targets of the I-BET-762 bromodomain inhibitor, was negatively correlated with *NNMT* expression ($r = -0.319$, $p = 2.87 \times 10^{-24}$; Table 1 and Supplementary

Table 1). Expression of multiple histone deacetylase (HDAC) genes showed both positive and negative weak correlations with numerous OCM genes ($|r|$ between 0.301 and 0.353, $p \leq 4.58 \times 10^{-23}$). Among positive correlations was expression of *HDAC1* with *SHMT1, DHFR,* and *MTHFD1,* that of *HDAC2* with *TYMS, HDAC3* with *DHFR, HDAC10* with *FTCD,* and *HDAC11* with *SLC46A1.* Negative correlations were observed between *HDAC5* and *MTHFS,* and between *HDAC11* and *GART* and *TYMS.* Products of these HDAC genes are targeted by histone deacetylase inhibitors tubastatin A, vorinostat, and VNLG/124. Sensitivity to those agents was significantly ($p \leq 2.14 \times 10^{-12}$) correlated with increased expression of *GART* ($r = -0.321$), *NNMT* ($r = -0.324$), and *SHMT2* ($r = -0.332$), respectively (Table 1; Supplementary Table 1). Association of OCM gene expression with sensitivity to HDAC inhibitors may be due to multiple direct and indirect mechanisms that include correlations between OCM gene expression and *HDAC* genes, as well as multiple metabolic and regulatory links between the OCM reactions and processes targeted by the HDAC inhibitors [7,77]. For example, NNMT directly controls the ratio of SAM to *S*-adenosyl homocysteine (SAH), and cellular NNMT levels and its activity directly affect histone methylation and the activity of DNA methyltransferases and histone methyltransferases [77]. Additionally, treatment with HDAC inhibitors directly inhibits glucose transport and results in inhibition of glycolysis, which is linked to the OCM cycle via the serine synthesis pathway (SSP) [1,77,100].

Interestingly, we observed strong significant associations of multiple OCM genes with expression of the *BRCA1* gene, which is involved in homologous recombination repair (Table 3; Supplementary Table 4). These correlations were positive for *DHFR* (Pearson $r = 0.608$), *TYMS* ($r = 0.485$), *GART* ($r = 0.427$), *MTHFD1* ($r = 0.408$), *SHMT1* ($r = 0.332$), *ATIC* ($r = 0.331$), and *SLC19A1* ($r = 0.318$) and moderately negative for *NNMT* ($r = -0.352$; FDR adjusted $p \leq 4.17 \times 10^{-24}$ for all genes listed). Positive correlations of many OCM genes with *BRCA1* expression are consistent with a previous report that cell line treatment with folic acid resulted in an increased *BRCA1* gene expression in many cell lines; however, that study found that this association had no effect on DNA repair or that such effects were transient [101]. The biological impact of positive correlations between expression levels of OCM genes and increased *BRCA1* expression on drug sensitivity is unclear, because elevated expression of several OCM genes in the GDSC-CCLE dataset also showed very weak ($|r| < 0.3$) but statistically significant (FDR adjusted $p < 0.05$) associations with sensitivity to multiple PARP inhibitors. For example, *TYMS* had $r = -0.273$ for correlation with log(IC50) of olaparib and $r = -0.261$ with ABT-888 (veliparib), *SHMT2* had $r = -0.288$ with ABT-888, and *MTR* had $r = -0.258$ with BMN-673 (talazoparib). In contrast, *NNMT* showed no association with log(IC50) of PARP inhibitors, whereas increased expression levels of a folate receptor gene, *FOLR1,* and a folate transporter gene, *SLC46A1,* were very weakly associated with resistance to PARP inhibitors (e.g., $r = 0.278$ for correlation of *SLC46A1* expression with ABT-888). The opposing directions of these weak correlations of OCM gene transcriptional levels with response to PARP inhibitors suggest the need for further investigation of any possible biological consequences of strong correlations between OCM gene expression and *BRCA1* gene expression.

OCM gene expression was a significant predictor of cancer cell line response to crizotinib when accounting for genomic alterations affecting crizotinib sensitivity

Sensitivity to certain kinase inhibitors is known to be strongly associated with, or in a number of cases require the presence of specific genomic alterations including gene amplifications, genome rearrangements, or specific protein-changing mutations; in addition, initially sensitive tumors often acquire secondary mutations that result in drug resistance (Supplementary Table 5) [51,54,79]. We examined three kinase inhibitors, erlotinib, lapatinib, and crizotinib (Table 1), for which sensitivity and resistance within specific cancer categories have been associated with specific genomic rearrangements, gene amplification, or with DNA and protein sequence changes in their molecular target genes (Supplementary Table 5). The numbers of cell lines with drug response and gene expression data that also had genomic alterations associated with sensitivity to erlotinib or lapatinib were insufficient (≤ 1) to account for such genomic changes (Supplementary Table 5). We were able to analyze cell line response to crizotinib across all cancer categories, conditional on the presence of genomic alterations affecting crizotinib sensitivity (*ALK* fusions, *ROS1* fusions, *MET* amplification, or *MET* exon 14 skipping mutations) or promoting resistance to that agent (Supplementary Table 5). As discussed above, expression of *MAT2B, MTR, SLC46A1*, and *SHMT2* was correlated with crizotinib response (Table 1). In multiple regression analyses, pre-treatment expression of each of these OCM genes remained a significant predictor of log(IC50) of crizotinib after accounting for the presence of genomic alterations with known roles in crizotinib sensitivity or resistance (FDR adjusted p between 1.38×10^{-6} and 0.0084 for $\log_2$ of OCM gene expression; Supplementary Table 6). The presence of genomic alterations affecting crizotinib sensitivity was also significantly associated with crizotinib response (p between 0.0121 and 0.0469; Supplementary Table 6). Overall, the fit of the multiple regression models was poor (Supplementary Table 6), most likely due to the low number of cell lines with relevant genome alterations, which dictated the combined use of data from a variety of cancer categories. None of the four OCM genes, *MAT2B, MTR, SLC46A1*, and *SHMT2,* had significant differences in $\log_2$ of their expression values between the groups of cell lines with and without genomic alterations known to affect sensitivity to crizotinib (p between 0.3126 and 0.6149 when using the Student's t-test; data not shown). Therefore, OCM gene expression was a significant predictor of crizotinib response independently from the status of genome alterations known to affect crizotinib sensitivity.

Discussion

Our analysis of correlations among OCM gene expression in tumor cell lines showed that expression levels of many OCM genes were correlated with each other, suggesting their possible co-regulation (Table 2). We also observed multiple modest associations of OCM gene expression with tumor cell response to individual antitumor agents in the GDSC-CCLE dataset (Table 1). While some of these associations confirm

several previously published reports [12,82], given the comparatively weak correlations with drug response (Pearson $|r|$ and Spearman $|\rho|$ between 0.3 and 0.4), they should be interpreted with caution and require further validation and investigation of their underlying molecular mechanisms. When six of the top 20 associations were examined in the NCI-60 dataset, the direction of all correlations was consistent with that in the GDSC-CCLE dataset, with higher expression levels of OCM genes being associated with drug sensitivity (Supplementary Table 2). However, only three out of six of these associations were statistically significant when using Pearson correlation analysis of the full NCI-60 dataset. The *NNMT*-dasatinib association was also significant when comparing the highest and the lowest *NNMT*-expressing cell lines, in agreement with a previously published report [82]. This association is of particular interest given the bimodality of *NNMT* expression (Fig. 2C).

The antitumor agents for which cell line sensitivity was modestly associated with elevated expression of multiple OCM genes may be of particular interest because this could suggest a possibility that they may be more dependent on OCM. Fig. 3 shows four genes, *GART, TYMS, SHMT2*, and *MTR,* for which higher expression levels were significantly associated with an increased sensitivity to 37 agents. Products of these OCM genes have unique functions. SHMT2 participates in the mitochondrial conversion of serine to glycine, which is accompanied by the mitochondrial production of 5,10-methyleneTHF from THF [1,7,8,28]. TYMS catalyzes the conversion of 5,10-methylene-THF to DHF [8,85,95]. This reaction is coupled with DNA synthesis, and during the *S* phase TYMS is transferred from the cytosol to the nucleus to provide deoxythymidine triphosphate for DNA replication [1,7,8]. GART catalyzes multiple important steps in purine biosynthesis while generating cytosolic THF from 10-formylTHF [20,28,96], whereas MTR participates in homocysteine remethylation to methionine using 5-methylTHF [20,85]. While all of these enzymes catalyze important reactions in the OCM pathway, their roles are diverse.

Twenty-two of the 37 agents shown in Fig. 3 were associated with two or more of the four genes, *GART, TYMS, SHMT2*, and *MTR*. Many agents in Fig. 3 are kinase inhibitors, which is consistent with associations of multiple kinases and kinase ligands with OCM gene expression (Table 3), indicating that the action of some kinases or their ligands targeted by these agents may be directly or indirectly associated with OCM reactions. Because higher expression levels of *GART, TYMS, SHMT2*, and *MTR* were associated with increased sensitivity to the agents listed in Fig. 3, this may suggest that cancer cells harboring higher levels of OCM reactions catalyzed by the products of these genes may be more sensitive to these agents. Among possible explanations for such associations could be either non-drug-specific sensitivity of cancer cells with higher levels of OCM reactions to drug treatment, or a possibility that molecular targets of such drugs might be associated with higher levels of expression of OCM-related genes in cancer cell proliferation and survival.

A broad explanation for the association of elevated OCM gene expression with sensitivity to various agents listed in Table 1 may involve an established association of increased levels of one-carbon metabolism reactions in tumor cells with higher rates of cancer cell proliferation, increased aerobic glycolysis, NADH production, and elevated levels of TYMS-mediated pyrimidine metabolism [8]. It may be possible to suggest that a combination of such processes in rapidly growing cancer cells could lead to small increases in the overall sensitivity of cancer cells to treatment with a variety of agents, resulting in modest associations listed in Table 1. Additionally, as discussed above, multiple genes whose products catalyze OCM reactions and a number of kinase genes may have elevated transcriptional levels in tumor cells [97,99], and the correlation of elevated rates of OCM reactions with higher kinase expression and associated drug sensitivity could indirectly result from progression of tumorigenesis. While such non-specific association of OCM genes with expression and activity of kinase targets appears plausible, there could also be a possibility of more direct connections between the activity or expression of individual kinases and the genes involved in folate metabolism and transport. For example, one study [103] noted increased ERBB2 protein expression in mammary tumors of rats who received folate supplementation. In addition, some reports [1,102] suggested regulation of transcriptional levels of *PHGDH* via ERBB2 overexpression, as well as possible regulation of SLC19A1 activity by phosphorylation, which could possibly indicate a regulatory effect of kinase activity on SCLC19A1; notably, activities of both PHGDH and SLC19A1 contribute molecular components to OCM reactions. Therefore, it may be possible that association of OCM gene expression with expression of multiple kinase genes could result from a combination of indirect and direct causes.

Another possible explanation for associations of drug response to diverse antitumor agents with expression of OCM genes may involve common mechanisms of gene regulation or activation. For example, the role of the E2F family of transcriptional regulators as a possible common mechanism in co-regulation of OCM genes had been discussed previously [94]. The mammalian target of rapamycin, mTOR, is a common regulator of one-carbon metabolism that can influence the expression of OCM genes and of components of the serine synthesis pathway thorough its action on transcriptional factors ATF4 (the activating transcription factor 4) and FOXK1, with additional feedback signaling from the OCM cycle affecting mTOR, as the mTORC1 complex is directly regulated by SAM, which is synthesized in the OCM pathway [2,7,20]. Another possible common mechanism could involve *c*-Myc and/or HIF-1, which regulate the components of various metabolic processes including the tricarboxylic acid (TCA) cycle and OCM reactions and enhance cancer cell proliferation [1,17,28,104]. HIF-1 and *c*-Myc play important roles in regulation of glycolysis, in the control of multiple enzymes involved in serine biosynthesis and catabolism, and in regulation of other metabolic processes in cancer cells that are connected with OCM reactions [2,28,77]. They promote *SHMT2* expression, and *c*-Myc also regulates *SHMT1* expression and enhances expression of nucleotide biosynthesis-related genes [1,2,28]. While both *c*-Myc and HIF-1 upregulate expression of certain OCM genes [2,28], expression levels of many OCM genes were correlated (Table 3), suggesting their possible concerted co-regulation. This could affect biological processes that may be regulated by or interfere with the action of the targets of the drugs associated with OCM gene expression. A drug blocking the transcription or protein activity of *c*-Myc and HIF-1 may be able to induce cell death, and cancer cells harboring higher OCM gene expression might be

more sensitive to such agents. For example, in addition to the role of mTOR in regulation of the OCM pathway, there have been multiple reports of *c*-Myc and HIF-1 activity and protein stability being regulated by the PI3K/AKT/mTOR pathway [105–110]. In support of this hypothesis, *SHMT2* expression was weakly ($r=0.333$, FDR adjusted $p=1.85\times10^{-26}$) associated with expression of *PDK1*, the target of the PI3K/AKT/mTOR inhibitor BX-912 (Supplementary Table 4), and sensitivity to that agent was associated with expression of both *MTR* and *TYMS* ($r=-0.307$ and -0.363, respectively; Table 1). *c*-Myc and HIF-1 may also be regulated by other targets of agents associated with OCM gene expression (Table 1). For example, p38 MAPK signaling targeted by TAK-715 and VX-702 [25,39,47,58] was reported to be essential for HIF-1 activation [111]. CaMK2 targeted by XMD15-27 [25] induces *c*-Myc protein stabilization and activation of HIF-1 [112,113], whereas SIRT1 targeted by EX-527 [25,39] induces *c*-Myc and HIF-1 activation [114,115]. *c*-Myc and HIF-1 activate DNA repair and TCA metabolism [19,116,117], which may involve additional agents shown in Fig. 3. Based on these considerations, *c*-Myc and HIF-1 could affect folate metabolism levels in cancer cells with increased proliferation, and it may be possible to speculate that such cells may be sensitive to the drugs inhibiting the *c*-Myc and HIF-1 pathways. However, our analysis revealed only a small number of OCM genes which showed both statistically significant and modest correlations (Pearson $|r|>0.3$) with *MYC* and *HIF1A* expression (Supplementary Table 4). For *HIF1A,* they included *GART* ($r=-0.346$, FDR adjusted $p=1.10\times10^{-28}$) and *NNMT* ($r=0.330$, $p=5.53\times10^{-26}$). For *MYC,* these genes were *SHMT2* ($r=0.307$, FDR adjusted $p=1.99\times10^{-22}$) and *SLC19A1* ($r=0.346$, $p=1.08\times10^{-28}$), with *GART* also showing a significant correlation with *r* close to 0.3 ($r=0.294$, FDR $p=1.37\times10^{-20}$). While *c*-Myc and HIF-1 protein activity may not be directly associated with the levels of expression of their genes, *MYC* and *HIF1A*, however, positive associations of *MYC* expression with *SHMT2* and *GART* expression may support the hypothesis of possible *c*-Myc involvement in associations between expression of these OCM genes and drug response. In contrast, a significant negative correlation between *GART* and *HIF1A* gene expression may indicate that HIF-1-induced transcriptional regulation of *GART* could be influenced only by post-transcriptional mechanisms, or that factors other than HIF-1 may have a direct positive effect on *GART* overexpression in tumors.

Among other possible contributors to drug sensitivity, cellular response could be affected via separate drug-specific mechanisms associated with OCM gene activity and function, with possible contributions from the serine metabolism pathway and the pentose phosphate pathway, AMP-activated protein kinase activity, mTORC1 function, the effects of the OCM reactions and the methionine cycle on cellular methylation processes, or via other mechanisms [1,8]. As discussed above, the activity of epigenetic factors and of epigenetic modifications and metabolic processes which are targeted by the HDAC inhibitors is directly linked to the OCM cycle [1,77,100]. *TYMS* and *DHFR* are among the most overexpressed genes in tumor cells, and their upregulation, as well as overexpression and amplification of *SHMT2* in cancer cells, are important contributors to the growth and survival of cancer cells [20,118]. Further investigation may be needed of how the interplay between the action of individual drugs and OCM gene expression may be affected by the genetic background of cancer cells, e.g. by the presence of *KRAS* mutations which may elevate OCM activity [1], by the *LKB1* and *PKCζ* deletion status and p53 mutation status [8] or by other sequence variants associated with OCM gene expression [119].

Our analysis of cell line response to crizotinib suggested that OCM gene expression was associated with response to crizotinib independently from the presence or absence of genomic alterations affecting sensitivity to that agent. This analysis combined the data across different cancer categories, as the number of genomic alterations was low and the cell lines carrying these alterations belonged to different histologies (non-small cell-lung cancer, stomach adenocarcinoma, and breast cancer). Future studies of specific cancer categories with sufficiently large sample sizes may be able to examine the effect of OCM gene expression on drug response in specific cancer types, while accounting for functional genome alterations and expression of target kinase genes.

Although the observed associations were modest, it may be appropriate to investigate whether pretreatment levels of OCM genes could be of potential use for selection of associated agents in Table 1 for cancer treatment, possibly in combination with additional information about cancer-related genomic alterations in non-OCM genes that would further assist in therapy choices. While higher baseline expression levels of many genes presented in Table 1 and in Fig. 3, e.g. *GART, TYMS, SHMT2, MTR, BHMT, MAT2B,* and *MTHFD2,* have been associated with increased *in vitro* sensitivity to multiple agents in our study and in other reports [12], a number of clinical studies have reported an association of elevated expression of these genes with poorer patient survival [69,88–92]. Some of such clinical associations involved expression changes in OCM genes in response to therapy [89] as opposed to pretreatment transcriptional levels examined in this study; however, other studies involved treatment-naïve patients [91]. Some pretreatment differences in OCM gene expression in the clinical setting may be associated with cancer subtypes, which had been previously demonstrated in ALL patients [94]. There could also be additional explanations for the differences between the associations of OCM gene expression with *in vitro* drug response and with clinical response. In addition to possible physiological effects of clinical dosing, drug metabolism, toxicity, and patient immune response, there are possible molecular explanations including known OCM-related drug response differences and cellular vulnerabilities between *in vitro* cancer cell lines and proliferating *in vivo* tumors that are related to the differences between the nutrient content of the tumor microenvironment and of tissue culture media [20]. As an example of such differences, the tumor microenvironment has low serine availability. In contrast, cultured cancer cells provide one-carbon units to the OCM cycle from serine but not from glycine, and they also lack homocysteine remethylation, due to the presence of excess methionine and the absence of cobalamine in standard tissue culture media, which also has other alterations in the nutrient content when compared to body fluids [20,120]. If the direction of *in vitro* associations with drug sensitivity observed in our study and by others [12] is validated by future studies, additional *in vivo* studies would be needed to confirm the clinical utility of these correlations. To examine the validity of these molecular associations *in vivo,* it may be useful to use mouse

PDX models to examine treatment response of tumors with increased or decreased levels of expression of specific OCM genes. It may be appropriate to use such models to examine associations of OCM gene expression levels prior to treatment and during the course of treatment with various measures of *in vivo* response to agents listed in Table 1, including tumor volume, rates of growth, and other measures of disease progression. If the results of mouse PDX studies are consistent with findings from cancer cell line screens, further clinical analyses of pretreatment expression levels of OCM genes in patients from specific cancer groups and of changes in gene expression during treatment may assist with determining whether any genes reported in this study may be used as potential biomarkers for treatment decisions.

In summary, we found an association of expression levels of ten OCM genes with chemosensitivity or chemoresistance to multiple cancer treatment agents in cancer cell lines. Among these genes, elevated expression of *SLC46A1* was consistently associated with chemoresistance to a variety of agents, and that of *GART, TYMS, SHMT2, MTR, BHMT*, and *MAT2B* was associated with chemosensitivity to multiple drugs. Although the detected correlations were modest, our findings indicated that pretreatment expression levels of OCM components may be directly or indirectly associated with response of a number of antitumor agents with diverse mechanisms of action. If validated *in vivo*, these associations may need to be taken into account when considering cancer treatment regiments and drug combination strategies.

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Conflict of interest

The authors declare no competing interests with this research.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.05.005.

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