Published in final edited form as:
Mol Cancer Ther. 2010 December ; 9(12): 3105–3114. doi:10.1158/1535-7163.MCT-10-0674.

Genome-Wide mRNA and microRNA Profiling of the NCI 60 Cell Line Screen and Comparison of FdUMP[10] with fluorouracil, floxuridine, and Top1 Poisons

William H. Gmeiner[1], **William C. Reinhold**[2], and **Yves Pommier**[2]

[1] Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, North Carolina [2] Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland

Abstract

A profile of microRNA and mRNA expression patterns across the NCI-60 cell line screen was analyzed to identify expression signatures that correlate with sensitivity to FdUMP[10], fluorouracil (5FU), floxuridine (FdU), topotecan, and irinotecan. Genome-wide profile analyses revealed FdUMP[10] resembles FdU most closely and shows dissimilarities with 5FU. FdUMP[10] had the largest dynamic range of any of these drugs across the NCI-60 indicative of cancer cell-specific activity. Genes involved in endocytosis, such as clathrin (CLTC-1), SNF8, annexin A6 (ANXA6) and amyloid protein-binding 2 (APPBP2) uniquely correlated with sensitivity to FdUMP[10], consistent with a protein-mediated cellular uptake of FdUMP[10]. Genes involved in nucleotide metabolism were enriched for the three fluoropyrimidine drugs, with the expression profile for 5FU correlated to an RNA-mediated cytotoxic mechanism, while expression of glycosyltransferases (XYLT2) that utilize UDP-sugars as substrates and the nucleoside diphosphatase and metastasis suppressor NM23 (NME1) were associated with FdUMP[10] sensitivity. Topotecan and irinotecan had significant negative correlations with miR-24, a microRNA with a high aggregate P_{CT} score for Top1. Our results reveal significant new correlations between FdUMP[10] and Top1-poisons as well as new information on the unique cytotoxic mechanism and genomic signature of FdUMP[10].

Keywords

Topoisomerase; microarray; miRNA; Fluoropyimidines; FdUMP[10]; irinotecan; topotecan; 5-fluorouracil; floxuridine

INTRODUCTION

Microarray profiling provides insights into the cytotoxic mechanisms of anticancer drugs as well as genomic signatures associated with drug activity (1–3). A particularly powerful approach is correlation of the sensitivity of one or more drugs to gene expression profiles across a collection of cells lines, such as the NCI-60 cell line screen, for which drug

Corresponding Author: William H. Gmeiner, Department of Cancer Biology, Wake Forest University School of Medicine, Winston-Salem, NC 27157. Phone 336-716-6216; Fax 336-716-0255. bgmeiner@wfubmc.edu.

Potential Conflict of Interest: William H. Gmeiner has ownership in Salzburg Therapeutics which holds the licenses for commercialization of FdUMP[10].

sensitivities are well documented (4–6). While the general response (e.g. growth inhibitory 50 [GI_{50}] values) profiles across the NCI-60 cell line screen can indicate mechanistic similarities and differences between two or more drugs, the basis of the COMPARE approach (7,8), additional mechanistic insights can be obtained from analysis of gene expression signatures in concert with microRNA expression signatures (6).

The poly-fluoropyrimidine antitumor agent FdUMP[10] has shown promising activity in pre-clinical studies including strong activity towards malignancies (e.g. leukemia (9)) that are not responsive to traditional fluoropyrimidine (FP) chemotherapeutics, such as fluorouracil (5FU) and fluoxuridine (FdU) (Figure 1A). Compare analysis of FdUMP[10] across the NCI-60 cell line screen data revealed similarities between FdUMP[10] and the Top1 poisons, irinotecan and topotecan, and a very distinct cytotoxic profile from 5FU (9). Subsequent functional analysis showed that treatment of cancer cells with FdUMP[10] resulted in formation of Top1 cleavage complexes demonstrating that the observed correlations of drug sensitivity had mechanistic significance (9).

In this study, we present an analysis of mRNA and microRNA expression profiles that correlate with sensitivity to FdUMP[10], 5FU, FdU, topotecan and irinotecan (Figure 1A) across the NCI-60 cell line screen. Our results further demonstrate mechanistic similarities of FdUMP[10] with Top1 poisons based on expression profile similarities, while clarifying distinct mechanistic features of FdUMP[10] based on the unique mRNA and microRNA profile for this drug that is distinct from both alternative FPs (e.g. 5FU and FdU) as well as from other Top1 poisons (e.g. irinotecan and topotecan).

MATERIALS & METHODS

Cell Culture and RNA Purifications

All cell lines were obtained directly from the Developmental Therapeutics Program (DTP), Division of Cancer Treatment and Diagnosis (DCTD), and grown as described previously (5). In brief, cells were revived from frozen stocks, and passed two times prior to harvest to minimize potential passage number associated variation. Purifications and quality control for mRNA and microRNA were as described previously (5).

Drug activities

Drug activities were obtained from the Developmental Therapeutics Program (DTP) (http://dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp). They are graphed in Figure 1 as the mean-centered −log 10 values of 50% growth inhibition (as presented for a subset of the data in Table 1) as measured by a 48-hour sulphorhodamine B assay of total protein, which has been described previously (10,11).

Correlation analysis

All correlations appearing in Tables 2 through 5 are Pearson's, and were calculated in Excel 2008 for MacIntosh. Statistical significance for $n = 60$ is 0.254 at $p \leq 0.05$.

Transcript probe and probe set data

For the genes described in Table 3, the transcript expression levels were determined using the probes from five platforms. These included from Affymetrix (Affymetrix Inc., Sunnyvale, CA) the ~60,000 features Human Genome U95 Set (HG-U95) (12); the ~44,000 features Human Genome U133 (HG-U133) (12); the ~47,000 feature Human Genome U133 Plus 2.0 Arrays (HG-U133 Plus 2.0); and the ~5,500,000 feature GeneChip Human Exon 1.0 ST array (GH Exon 1.0 ST) (13). From Agilent (Agilent Technologies, Inc., Santa Clara, CA) we used the ~41,000 features Whole Human Genome Oligo Microarray (14).

Normalization of HG-U95 and HG-U133 was done by GCRMA (15). Normalization of HG-U133 Plus 2.0 and the Whole Human Genome Oligo Microarray was by RMA (16). Agilent mRNA probes detected in at least 10% of the cell lines were normalized using GeneSpring GX including i) setting gProcessedSignal values less than 5 to 5, ii) transforming gProcessedSignal or gTotalGeneSignal to Logbase 2, and iii) normalizing per array to the 75th percentile (14). HG-U95, HG-U133, and Agilent Whole Human Genome Oligo Microarray data can be accessed at CellMiner, at http://discover.nci.nih.gov.

Relative gene expression levels were determined based on the probes (Agilent) or probe sets (Affymetrix) that passed the following quality control criteria. Intensity ranges were determined for all probe sets (meant to include Agilent probes in the following text), and those with intensity range of greater or equal to 1.2 $\log_2$ were kept. The number of probe sets that passed this criterion was determined for each gene, and 25% of that number calculated. Pearson's correlations were next determined for all possible probe set combinations. Average correlations for each probe set were determined compared to all other probe sets for that gene. Probe sets with average correlations (to other probe sets) less than 0.30 were dropped. For those genes with probe sets with average correlations less than 0.60, the probe set with the lowest correlation was dropped and the correlations recalculated for the remaining possible probe set/probe set combinations. The probe sets with the lowest average correlations continued to be dropped, and the average recalculated until either all average correlations were ≥ to 0.60, or the 25% of the original probe set number (calculated above) was reached.

Determination of Z scores

To obtain single composite values of the probe set intensities that passed quality controls criteria, intensities were transformed to z-scores (http://en.wikipedia.org/wiki/Standard_score) by subtracting their 60 cell line means, and dividing by their standard deviations. We then determined the average z-scores for all available (16,820) genes. These calculations were done in Java.

Determination of microRNA expression levels

We have described previously the purification, quality assessment, and expression level determinations of the microRNA shown in Table 4 (14). In brief, total RNA (100 ng) was labeled following the recommendations of Agilent Technologies (miRNA Microarray System Protocol v 1.5) and hybridized to the Agilent Technologies Human miRNA Microarray (V2). Scanning and data extraction of the arrays was done as recommended by Agilent Technologies. This expression data is available at http://discover.nci.nih.gov/cellminer/.

RESULTS AND DISCUSSION

Sensitivity/Resistance Profiles

The relative sensitivity/resistance profile for a drug over the 60 cell lines included in the NCI screen provides valuable information that can group drugs based upon a common mechanism of action and also may indicate which malignancies are most sensitive, and thus most likely to respond to a given drug. The relative sensitivity/resistance profile for FdUMP[10] is distinct from that of 5FU consistent with these two fluoropyrimidine (FP) drugs having distinct cytotoxic mechanisms (Figure 1B). While the sensitivity/resistance profile for FdUMP[10] more closely resembles FdU than 5FU, distinct differences between these two types of FPs are also evident in the sensitivity/resistance data and profile (Figure 1).

The sensitivity profile for FdUMP[10] has several features that distinguish it from other FP drugs and other cytotoxic compounds, as well. A class of malignancy that is more sensitive than average to FdUMP[10], on aggregate, yet traditionally not treated with FP drugs are renal malignancies (Figure 1B). The overall responsiveness of cells included in the NCI-60 cell line screen to FdUMP[10] was much greater than for 5FU, FdU, and topotecan, and the average responsiveness was comparable to irinotecan (Figure 1C and Table 1). Interestingly, the dynamic range of GI_{50} values for FdUMP[10] was four orders of magnitude, which greatly exceeds the other drugs included in this study (Figure 1C and Table 1). The robust 10,000 fold dynamic range of FdUMP[10] provides two advantages. The first is that it extends the activity of the drug above all of the other drugs in this study, on average by 1.48 $\log_{10}$ (with a range of 0.23 to 2.32). The second is that it provides a potential treatment rationale. That is, one might use markers to recognize those tumors with high sensitivity to FdUMP[10] to greater affect than the other drugs in this study, as they display less robust ranges that average 2.70 $\log_{10}$.

The overall sensitivity/resistance profile for FdUMP[10] resembles that for the Top1 poisons topotecan and irinotecan nearly as closely as it resembles FdU (Figure 1). Further, the sensitivity/resistance profile for FdUMP[10] is almost as similar to that of topotecan and irinotecan as these two Top1 poisons are related to one another. A summary of the Pearson correlation coefficients derived from the sensitivity/resistance profile data for FdUMP[10], 5FU, FdU, topotecan and irinotecan is shown in Figure 1D. SN-38, the active metabolite of irinotecan gave comparable results (not shown). The data are consistent with the cytotoxicity of FdUMP[10] being more strongly related to poisoning of Top1 than 5FU.

mRNA Positive Correlations

The broad dynamic range in the IC50 values across the 60 cell lines observed for FdUMP[10] provided a well-defined drug response profile, which could then be used for correlative analyses with the gene and micro-RNA database profiles of the NCI-60. Sensitivity to FdUMP[10] positively correlated with expression of hundreds of genes (with correlation coefficients greater than 0.4; $p < 0.01$) and we focused on the genes with known functions. Genes most highly correlated with FdUMP[10] sensitivity included clusters of genes important for endocytosis as well as genes involved in nucleoside metabolism, cell-cycle progression, induction of apoptosis and DNA repair. Tables of the genes most highly correlated with sensitivity to each of the drugs are included in the supplementary information together with a GO analysis identifying pathways correlated with drug sensitivity.

Genes Involved in Endocytosis—Previous studies have demonstrated that cells deficient in thymidine kinase (TK) display reduced resistance to FdUMP[10] relative to FdU and FdUMP, consistent with cellular uptake of FdUMP[10] occurring at least partially in multimeric form (17). The mechanism of cellular uptake of FdUMP[10] has not yet been determined. In the present analysis, sensitivity to FdUMP[10] was found to positively correlate with expression of proteins involved in endocytosis and intracellular transport. For example, FdUMP[10] sensitivity correlated positively with expression of CLTC ($r = 0.41$), SNF8 ($r = 0.42$), ANXA6 ($r = 0.42$), and APPBP2 ($r = 0.40$) (Table 2 and Figure 2).

CLTC encodes clathrin, which is important for cellular internalization via clathrin-coated pits (18). Cellular internalization via clathrin-coated pits is a potential mechanism for cellular internalization of FdUMP[10], and cells expressing elevated CLTC levels would be expected to efficiently internalize FdUMP[10] via a clathrin-dependent mechanism. CLTC also had a positive correlation with FdU ($r = 0.29$). However CLTC1 had a negative

correlation with 5FU (r = −0.25). Neither CLTC nor CLTC1 were significantly correlated to topotecan or irinotecan.

FdUMP[10] also correlated with SNF8, a component of the endosomal sorting complex, ESCRT-II, which plays an important role in cellular uptake and sub-cellular routing of internalized proteins (19). SNF8 tends to have the lowest expression in the breast carcinoma cells (Figure 2). It was not correlated with any of the other four drugs evaluated.

ANXA6 encodes annexin A6, and although the function of this protein has not yet been fully determined, other annexin family members have been implicated in membrane-related events including endocytosis (20). ANXA6 expression also correlated with topotecan (r = 0.32).

Amyloid protein-binding protein 2 is encoded by the APPBP2 gene. It is associated with protein transport, particularly as it relates to beta-amyloid transport, and may have a more generalized role in cellular transport. Although APPBP2 and has been found highly expressed in breast and ovarian cancer (21), Figure 2 shows that MCF7 cells exhibit very high expression of APPBP2, whereas APPBP2 expression does not appear selectively high in the other breast and ovarian cell lines of NCI-60.

The identification of genes involved in cellular internalization is consistent with a cytotoxic mechanism of FdUMP[10] involving cellular internalization via endocytosis, and the correlations with topotecan and irinotecan with a DNA-directed mechanism of action including the occurrence of Top1-induced DNA damage. The fact that more genes involved in endocytosis and sub-cellular routing correlate with FdUMP[10] sensitivity than with sensitivity to any of the other four drugs analyzed is consistent with uptake of FdUMP[10] occurring by a protein-mediated process in many cell types.

Genes involved in Nucleoside Metabolism—A potential advantage of FdUMP[10] relative to currently used FP drugs, such as 5FU, is that fewer steps of metabolic activation are required to produce FdUMP and FdUTP, the DNA-directed FP metabolites that are responsible for antitumor activity (9,22). A summary of correlation of expression of genes important for nucleotide metabolism with drug sensitivity is included in Table 3. Somewhat surprisingly, the TYMS gene encoding thymidylate synthetase (TS) (23) was not strongly correlated with FdUMP[10] sensitivity, but was for irinotecan (r = 0.35), topotecan (r = 0.30), and FdU (r = 0.26). TS expression also did not correlate with 5FU sensitivity. The lack of correlation of FdUMP[10] sensitivity with TS expression likely results from the high efficiency of TS inhibition with FdUMP[10] treatment (24) while the correlation of TS expression with Top1 poisons may reflect a correlation with an elevated proliferation rate. Uridine monophosphate synthetase (UMPS) (25), did however, correlate with 5FU sensitivity indicating 5FU conversion to ribonucleotides is important for 5FU-mediated cytotoxicity, a finding consistent with 5FU being mainly an RNA-mediated drug (26). There was no correlation of 5FU sensitivity with ribonucleotide reductase. Dihydropyrimidine dehydrogenase (DPYD) was found to correlate negatively with 5FU sensitivity (r = −0.34) consistent with DPYD degradation of 5FU limiting the biological effects of this drug (27). There was no correlation of DPYD expression with sensitivity to either FdUMP[10] or FdU. Genes expressing thymidine kinases (e.g. DTYMK) were not found to correlate with sensitivity to any of the five drugs. While thymidine kinase deficiency results in resistance to FdU, but not FdUMP[10] (17), the lack of correlation with FdU sensitivity probably indicates a moderate range in DTYMK expression across the 60 cell lines with relatively few highly deficient cells in DTYMK expression.

The NME1 gene (28) that encodes nucleoside diphosphate kinase A correlated with all five drugs with the strongest correlation for FdUMP[10] (r = 0.47) and the weakest correlation for 5FU (r = 0.27) (Table 3). NME1 is also known as the metastasis suppressor NM23 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=NME1&search=nme1) (29). NME1 expression appears to vary widely across cell lines irrespective of their tissue of origin (Figure 2).

Xylosyltransferase 2 (XYLT2) (30), a glycosyltransferase that transfers xylose from UDP-sugars to serine residues of proteins, correlated with sensitivity to FdUMP[10] (r = 0.44) (Table 3). XYLT2 appears consistently low in leukemias (Figure 2) and did not significantly correlate with sensitivity to either FdU or 5FU (Table 3). Interestingly, O-linked N-acetyl glucosamine (GlcNAc) transferase (OGT) (31) negatively correlated with FdUMP[10] sensitivity (r = −0.26), and has overall high expression in the leukemias (Figure 2), but did not correlate with either 5FU or FdU (Table 3). Thus, genes involved in nucleoside metabolism, including glycosyl transferases that use UDP-sugars as substrates, contribute to FdUMP[10] sensitivity. Importantly, the spectrum of genes involved in nucleoside metabolism that correlate with FdUMP[10] sensitivity is distinct from that of 5FU.

Topoisomerase I—Previous studies have demonstrated that treatment of cancer cells with FdUMP[10] results in formation of Top1 cleavage complexes (9). Further, cancer cells resistant to Top1 poisons, such as topotecan and irinotecan, also display a degree of cross-resistance to FdUMP[10]. The sensitivity/resistance profile for FdUMP[10] is more highly correlated with Top1 poisons than with 5FU (Figure 1B & D). Sensitivity to topotecan and irinotecan correlates only weakly with TOP1 expression (data not shown) (32–34). The weak correlation of topotecan and irinotecan with TOP1 expression likely results from regulation of Top1 protein levels by ubiquitin-mediated degradation rather than from altered expression levels (32). Other topoisomerases and related proteins that correlated significantly with topotecan sensitivity included TOP2A (r = 0.29) and TOP3A (r = 0.26). Similarly for irinotecan, sensitivity correlated significantly with the expression of TOP1P2 (the TOP1 pseudogene 2) (r = 0.34), TOP1 (r = 0.28), TOP3A (r = 0.33), and TOP2A (r = 0.26).

DNA Damage & Repair—Treatment of cancer cells with FdUMP[10] results in substantial DNA damage with DNA double-strand breaks (DSBs) initially evident approximately 16 h following drug treatment (Gmeiner et al; unpublished data). Formation of Top1-induced DSBs is a major cause of the cytotoxicity for topotecan and irinotecan (35). The expression of several proteins important for DNA repair correlated with sensitivity to the drugs analyzed. Rad51C expression correlated with sensitivity to topotecan (r = 0.40), FdUMP[10] (r = 0.39), FdU (r = 0.31), irinotecan (r = 0.29) and also 5FU (r = 0.29). BRCA2 expression correlated significantly with sensitivity to FdU (r = 0.27) and 5FU (r = 0.32). Irinotecan sensitivity correlated significantly with BRCA1 expression (r = 0.29), PARP11 expression (r = 0.27), and ERCC8 (CSA) expression (r = 0.33). ERCC6 (CSB) expression had significant negative correlation with FdUMP[10] sensitivity (r = −0.29) and 5FU sensitivity (r = −0.36). Base excision repair (BER) proteins (UNG and TDG) correlated exclusively with 5FU sensitivity (r = 0.34, 0.34) and did not correlate with either FdU or FdUMP[10]. Thus, proteins important for DNA repair correlate with sensitivity to FdUMP[10] and the other drugs analyzed in this study.

mRNA negative correlations

Sensitivity to FdUMP[10] was also significantly negatively correlated with hundreds of genes; 430 genes having a correlation coefficient less than −0.254, and 13 of these displaying a correlation coefficient less than −0.4. Two genes involved in TGF signaling

(TGFBR3 and SMAD6) had significant negative correlation with sensitivity to FdUMP[10], as did three members of the anti-apoptotic Bcl2 family (BCL2L1, BCL2L2, and BCL2L11). These findings indicate down-regulation of pro-survival proteins sensitizes cells to FdUMP[10]. The gene-class that displayed the greatest overrepresentation among genes whose down-regulation was associated with sensitization to FdUMP[10] was the zinc-finger domain (ZNF) proteins. A total of 18 ZNF proteins were identified (ZNF440, ZNF558, ZNF763, ZNF69, ZNF587, ZNF473, ZNF700, ZNF20, ZNF175, ZNF787, ZNF321, ZNF460, ZNF600, ZNF703, ZNF320, ZNRF3, ZNF266, and ZNF649), twice as many as were identified among those genes whose up-regulation was associated with sensitization to FdUMP[10]. These results are consistent with proteins that interact with DNA via a zinc-finger motif as playing an important role in cellular sensitization to FdUMP[10].

microRNA correlations

The role of microRNA in regulating gene expression and its significance for cancer progression and treatment is being increasingly recognized (6). We performed a microRNA profile establishing to what extent microRNA up- or down-regulation was associated with cellular sensitivity to FdUMP[10] across the NCI-60 cell-line panel. Two microRNAs (miR-224 and miR-24-1*) had significant negative correlation with FdUMP[10] sensitivity (Table 4). mIR-224 is also negatively correlated to all five drugs with the highest correlation for FdUMP[10] (r= −0.32 for FdUMP[10], −0.18 for 5FU, −0.16 for FdU, −0.23 for topotecan and −0.18 for irinotecan). Among the genes implicated as being regulated by mIR-224 are apoptosis inhibitor 5 (36) and Rad54L2 (EMBL-EBI).

mIR-24-1* only correlated significantly with FdUMP[10] (r= −0.31 for FdUMP[10], −0.13 for 5FU, −0.25 for FdU, −0.17 for topotecan and −0.17 for irinotecan). mIR-24, which originates from the 3′-arm of the same hairpin as mIR-24-1* on chromosome 9 (http://www.mirbase.org/cgi-bin/mirna_entry.pl?acc=MI0000080) also negatively correlates with sensitivity to all five drugs (r = −0.24 for FdUMP[10], −0.31 for 5FU, −0.29 for FdU, −0.32 for topotecan, and −0.43 for irinotecan) (Table 4). Interestingly, Top1 is likely a target for regulation by miR-24 (Table 4). Elevated miR-24 potentially decreases Top1 protein reducing sensitivity to Top1 poisons. These results are consistent with Top1 activities, and hence sensitivity to Top1 poisons being regulated through expression of miR-24. Other genes that are putative targets for miR-24 include 5,10-methylenetetrahydrofolate reductase, DHFR (37), several Bcl2 transcript variants (BCl211 transcript variants 1, 6, 7, & 8), p53 inducible nuclear protein (TP53INP1), Ras p21 protein activator (RasA1), p27, and O-linked N-acetylglucosamine (GlcNAc) transferase. Among all the microRNAs examined, mIR-24 is the most negatively correlated with irinotecan sensitivity, and the mIR third most negatively correlated with topotecan and FdU sensitivity.

Regulation of the same genes that correlate with FdUMP[10] sensitivity by mRNA analysis also correlate with sensitivity based on miRNA expression including SMAD proteins (SMAD4 & SMAD5) and XYLT1 (Targetscan). FdUMP[10] sensitivity also has significant positive correlation with expression of 29 microRNAs (r > 0.25). A summary of the highest positive microRNA correlations for FdUMP[10] is included in Table 4.

CONCLUSIONS

Analysis of the sensitivity profile for FdUMP[10] across the NCI-60 cell line screen revealed FdUMP[10] is a potent compound with a unique sensitivity profile that differs markedly from traditional FPs and exhibits similarities to the Top1 poisons topotecan and irinotecan. The sensitivity profile for FdUMP[10] has the least correlation to 5FU among the five drugs reviewed in this study (Figure 1C), accentuating mechanistic differences between FdUMP[10] and 5FU. The average GI50 values also were consistent with strong

mechanistic differences between FdUMP[10] and 5FU. The average GI50 value for FdUMP[10] in the NCI 60 cell line screen is 7.1×10^{-8} M, which is 324-fold less than 5FU (average GI50 = 2.3×10^{-5} M). The potency of FdUMP[10] also greatly exceeds the stoichiometric content of the FdU components of the multimer.

The expression profiles for the genes important for nucleoside metabolism across the NCI-60 cell line panel reinforces these mechanistic dissimilarities between 5FU and FdUMP[10]. Sensitivity to 5FU correlates positively with expression of UMPS and negatively with expression of DPYD, consistent with 5FU conversion to ribonucleotide metabolites as being important for 5FU sensitivity, and with degradation of the nucleobase being detrimental to drug activity. Expression of UMPS and DPYD did not significantly correlate with FdUMP[10] activity, nor did expression of these genes correlate with FdU sensitivity. It is important to note that while both FdUMP[10] and FdU are DNA-directed FPs in tissue culture based on the expression profile analysis, that i) the glycosidic bond of FdU is readily cleaved *in vivo*, ii) FdU is readily converted to 5FU in peripheral blood mononuclear cells (38) and iii) FdU is not a DNA-directed FP (39). In contrast, FdUMP[10] is not a good substrate for glycosylases. Expression profiling analysis shows significant correlations with clathrin expression indicating that protein-mediated uptake of the multimer is important for FdUMP[10] activity.

In summary, we have undertaken a comparative genome-wide analysis of the determinants that underlie sensitivity profiles for five drugs (FdUMP[10], 5FU, FdU, topotecan, and irinotecan) across the NCI-60 cell line panel. The activity of FdUMP[10] across the NCI panel demonstrates a high dynamic range for FdUMP[10] across the NCI-60 cell line panel, which exceeds the other drugs examined. A number of genes and microRNA have been uncovered, which could form a basis for the rational development of FdUMP[10] as a novel anticancer agent and correlating drug response with genomic characteristics of individual tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial Support: This work was supported by NIH-NCI CA102532 (WG) and by the NIH Intramural Program, National Cancer Institute, Center for Cancer Research (Z01 BC 006161-17LMP).

References

1. Lamb J, Crawford ED, Peck D, et al. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006; 313:1929–35. [PubMed: 17008526]
2. Weinstein JN, Pommier Y. Connecting genes, drugs and diseases. Nat Biotechnol. 2006; 24:1365–6. [PubMed: 17093484]
3. Bonnefoi H, Underhill C, Iggo R, Cameron D. Predictive signatures for chemotherapy sensitivity in breast cancer: Are they ready for use in the clinic? Eur J Cancer. 2009; 45:1733–1743. [PubMed: 19477634]
4. Paull KD, Shoemaker RH, Hodes L, et al. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of a mean graph and COMPARE algorithm. J Natl Cancer Inst. 1989; 81:1088–92. [PubMed: 2738938]
5. Scherf U, Ross DT, Waltham M, et al. A gene expression database for the molecular pharmacology of cancer. Nat Genet. 2000; 24:236–44. [PubMed: 10700175]
6. Liu H, D'Andrade P, Fulmer-Smentek S, et al. mRNA and microRNA expression profiles of the NCI-60 integrated with drug activities. Mol Cancer Ther. 2010; 9:1080–91. [PubMed: 20442302]

7. Holbeck SL, Collins JM, Doroshow JH. Analysis of food and drug administration-approved anticancer agents in the NCI60 panel of human tumor cell lines. Mol Cancer Ther. 2010; 6:1451–1460. [PubMed: 20442306]
8. Zaharevitz DW, Holbeck SL, Bowerman C, Svetlik PA. COMPARE: a web accessible tool for investigating mechanisms of cell growth inhibition. J Mol Graph Pad. 2002; 20:297–303.
9. Liao Z-Y, Sordet O, Zhang H-L, Kohlhagen G, Antony S, Gmeiner WH, Pommier Y. A novel polypyrimidine antitumor agent FdUMP[10] induces thymineless death with topoisomerase I-DNA complexes. Cancer Res. 2005; 65:4844–4851. [PubMed: 15930305]
10. Boyd M, Paull K. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. Drug Development Research. 1995; 34:91–109.
11. Monks A, Scudiero D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst. 1991; 83:757–66. [PubMed: 2041050]
12. Shankavaram U, Reinhold W, Nishizuka S, et al. Transcript and protein expression profiles of the NCI-60 cancer cell panel: an integromic microarray study. Mol Cancer Ther. 2007; 6:820–32. [PubMed: 17339364]
13. Reinhold WC, Mergny JL, Liu H, et al. Exon array analyses across the NCI-60 reveal potential regulation of TOP1 by transcription pausing at guanosine quartets in the first intron. Cancer Res. 2010; 70:2191–203. [PubMed: 20215517]
14. Liu H, D'Andrade Petula, Fulmer-Smentek Stephanie, et al. mRNA and microRNA expression profiles integrated with drug sensitivities of the NCI-60 human cancer cell lines. MCT. 2010; 9(5): 1080–1091. [PubMed: 20442302]
15. Wu Z, Irizarry RA. Stochastic models inspired by hybridization theory for short oligonucleotide arrays. J Comput Biol. 2005; 12:882–93. [PubMed: 16108723]
16. Irizarry R, Bolstad B, Collin F, et al. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Research. 2003; 31(4):e15. [PubMed: 12582260]
17. Bijnsdorp IV, Comijn EM, Padron JM, Gmeiner WH, Peters GJ. Mechanisms of action of FdUMP[10]: metabolite activation and thymidylate synthase inhibition. Oncol Rep. 2008; 18:287–291. [PubMed: 17549381]
18. Henne MW, Boucrot E, Meinecke M, Evergren E, Vallis Y, Mittal R, McMahon HT. FCHo proteins are nucleators of clathrin-mediated endocytosis. Science. 2010; 328:1281–1284. [PubMed: 20448150]
19. Im YJ, Hurley JH. Integrated structural model and membrane targeting mechanism of the human ESCRT-II complex. Dev Cell. 2008; 14:902–913. [PubMed: 18539118]
20. Grewal T, Enrich C. Annexins – Modulators of EGF receptor signaling and trafficking. Cellular Signalling. 2009; 21:847–858. [PubMed: 19385045]
21. Hirasawa A, Saito-Ohara F, Inoue J, Aoki D, Susumu N, Yokoyama T, Nozawa S, Inazawa J, Imoto I. Association of 17q21-q24 gain in ovarian clear cell adenocarcinomas with poor prognosis and identification of PPM1D and APPBP2 as likely amplification targets. Clin Cancer Res. 2003; 9:1995–2004. [PubMed: 12796361]
22. Gmeiner WH. Genetic determinants for activated fluoropyrimidine chemotherapy. Drug Dev Res. 2006; 67:119–129.
23. Gmeiner WH. Novel chemical strategies for thymidylate synthase inhibition. Curr Med Chem. 2005; 12:191–202. [PubMed: 15638735]
24. Gmeiner WH, Trump E, Wei C. Enhanced DNA-directed effects of FdUMP[10] compared to 5-FU. Nucl Nuct Nucl Acids. 2004; 23:401–410.
25. Kim S, Park D-H, Kim TH, Hwang M, Shim J. Functional analysis of pyrimidine biosynthesis enzymes using the anticancer drug 5-fluorouracil in Caenorhabditis elegans. FEBS J. 2009; 276:4715–4726. [PubMed: 19645718]
26. Pritchard DM, Watson AJM, Potte CS, Jackman AL, Hickman JA. Inhibition by uridine but not thymidine of p53-dependent intestinal apoptosis initiated by 5-fluorouracil: Evidence for the involvement of RNA perturbation. Proc Nat Acad Sci. 1997; 94:1795–1799. [PubMed: 9050858]
27. Yen JL, McLeod HL. Should DPD analysis be required prior to prescribing fluoropyrimidines? Eur J Cancer. 2007; 43:1011–1016. [PubMed: 17350823]

28. Desvignes T, Pontarotti P, Fauvel C, Bobe J. Nme protein family evolutionary history, a vertebrate perspective. BMC Evolutionary Biology. 2009; 9:256–281. [PubMed: 19852809]
29. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. Nature Med. 2006; 12:895–904. [PubMed: 16892035]
30. Condac E, Silasi-Mansat R, Kosanke S, Schoeb T, Towner R, Lupu F, Cummings RD, Hinsdale ME. Polycystic disease caused by deficiency in xylosyltransferase 2, an initiating enzyme of glycosaminoglycan biosynthesis. Proc Nat Acad Sci USA. 2007; 104:9416–9421. [PubMed: 17517600]
31. Love DC, Krause MW, Hanover JA. O-GlcNac cycling: Emerging roles in development and epigenetics. Sem Cell Dev Biol. 2010 in press.
32. Pfister TD, Reinhold WC, Agama K, et al. Topoisomerase I levels in the NCI-60 cancer cell line panel determined by validated ELISA and microarray analysis and correlation with indenoisoquinoline sensitivity. Mol Cancer Ther. 2009; 8:1878–84. [PubMed: 19584232]
33. Goldwasser F, Bae IS, Torres K, Pommier Y. Topoisomerase I-related parameters and camptothecin activity in the colon cell lines from the National Cancer Institute anticancer screen. Cancer Res. 1995; 55:2116–21. [PubMed: 7743511]
34. Nieves-Neira W, Pommier Y. Apoptotic response to camptothecin and 7-hydroxystaurosporine (UCN-01) in the eight human breast cancer cell lines of the NCI anticancer drug screen: multifactorial relationship with topoisomerase I, protein kinase C, bcl-2 and caspase pathways. Int J Cancer. 1999; 82:396–404. [PubMed: 10399957]
35. Pommier Y. DNA topoisomerase I inhibitors: chemistry, biology, and interfacial inhibition. Chem Rev. 2009; 109:2894–902. [PubMed: 19476377]
36. Wang Y, Lee ATC, Ma JZI, Wang J, Ren J, Yang Y, Tantoso E, Li B-B, Ooi LLP, Tan P, Lee CGL. Profiling mircoRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-5 as a microRNA-22-specific target. J Biol Chem. 2008; 283:13205–13215. [PubMed: 18319255]
37. Mishra PJ, Song B, Mishra PJ, Wang Y, Humeniuk R, Banerjee D, Merlino G, Ju J, Bertino JR. MiR-24 tumor suppressor activity is regulated independent of p53 and through a target site polymorphism. PLoS One. 2009; 4:e8445 1–10. [PubMed: 20041160]
38. Temmink OH, de Bruin M, Laan AC, Turksma AW, Cricca S, Masterson AJ, Noordhuis P, Peters GJ. The role of thymidine phosphorylase and uridine phosphorylase in (fluoro)pyrimidine metabolism in peripheral blood mononuclear cells. Int J Biochem Cell Biol. 2006; 38:1759–1765. [PubMed: 16798057]
39. Brody JR, Hucl T, Costantino CL, Eshelman JR, Gallmeier E, Zhu H, van der Heijden MS, Winter JM, Wikiewicz AK. Limits to thymidylate synthase and TP53 genes as predictive determinants for fluoropyrimidine sensitivity and further evidence for RNA-based toxicity as a major influence. Cancer Res. 2009; 69:984–991. [PubMed: 19155291]

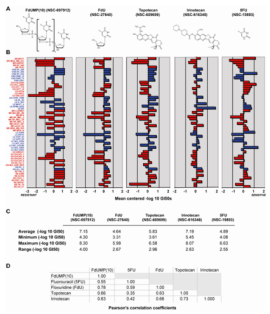

Figure 1.

A. Structures of the five drugs analyzed in the present study. **B**. Sensitivity profiles across the NCI-60 cell line screen. The sensitivity profile for FdUMP[10] significantly differs from that for 5FU indicating mechanistic difference for these drugs while the sensitivity profile for FdUMP[10] indicates similarities with the Top1 poisons topotecan and irinotecan. Topoisomerase 1 (Top1) is the sole target for topotecan and Irinotecan. Gene expression profiling reveals mechanistic similarities of FdUMP[10] with these Top1 poisons. **C**. Comparative potency (GI50 $-\log_{10}$ values) of the five drugs studied across the NCI-60. **D**. Correlation between drug activity profiles in the NCI-60. Pearson's correlation coefficient of 0.414 corresponds to a significant correlation ($p < 0.001$ in the absence of multiple comparisons correction – http://faculty.fortlewis.edu/CHEW_B/Documents/Table%20of%20critical%20values%20for%20Pearson%20correlation.htm).

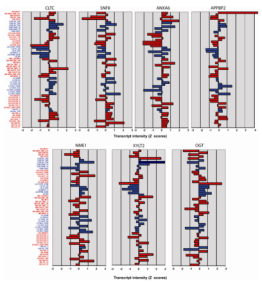

Figure 2.
Gene expression profiles across the NCI-60 for several genes significantly correlated with FdUMP[10] (see Tables 2 and 3 for further details).

Mol Cancer Ther. Author manuscript; available in PMC 2011 December 1.

Table 1

Drug Activities Across Leukemia and CNS Cell Lines and Summary Statistics for Complete NCI-60 Cell Line Screen

	FdUMP(10)	Fluorouracil (5FU)	Floxuridine (FUdR)	Topotecan HCl	Irinotecan HCl
NSC number	**697912**	**19893**	**27640**	**609699**	**616348**
CNS:SF-268	8.30	4.30	6.53	7.92	5.41
CNS:SF-295	8.30	4.30	6.50	7.69	5.14
CNS:SF-539	8.30	5.88	6.53	7.89	5.30
CNS:SNB19	6.77	3.94	5.91	7.66	4.62
CNS:SNB75	6.45	3.74	6.03	7.68	4.80
CNS:U251	6.94	4.35	6.35	7.79	5.39
LE:CCRF-CEM	na	4.48	6.41	8.07	5.46
LE:HL-60	7.90	4.68	6.27	7.85	4.95
LE:K-562	5.63	4.68	6.11	7.22	4.96
LE:MOLT-4	8.28	4.87	6.44	8.06	5.78
LE:RPMI-8226	6.64	5.31	5.74	6.61	4.69
LE:SR	8.30	5.38	6.50	7.66	6.63
NCI-60 Minimum	4.30	3.31	3.61	5.45	4.08
NCI-60 Maximum	8.30	5.98	6.58	8.07	6.63
NCI-60 Average	7.15	4.64	5.83	7.19	4.89
NCI-60 Range	4.00	2.67	2.96	2.63	2.55

[a] Drug activities are GI50's, presented as −log10 values.

[b] Drug activities obtained from http://dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp.

Table 2

Correlation of Selected Genes Implicated in Endocytosis

	FdUMP[10]	5FU	FdU	Topotecan	Irinotecan
CLTC	0.41	−0.25	0.29	nd[1]	nd
SNF8	0.42	nd	nd	nd	nd
ANXA6	0.42	nd	nd	nd	nd
APPBP2	0.40	nd	nd	nd	nd

[1] "nd" indicates that thecorrelation was less than cutoff (+/− 0.25).

Table 3

Correlation with Select Genes Implicated in Nucleoside Metabolism

	FdUMP[10]	5FU	FdU	Topotecan	Irinotecan
TYMS (TS)	nd[1]	nd	0.26	0.30	0.35
UMPS	nd	0.37	nd	nd	nd
DPYD	nd	−0.34	nd	nd	nd
NME1	0.47	0.27	0.35	0.33	0.35
XYLT2	0.44	nd	nd	0.29	nd
OGT	−0.26	nd	nd	nd	nd

[1]"nd" indicates that thecorrelation was less than cutoff (+/− 0.25).

Table 4

MicroRNA correlations

	FdUMP[10]	5FU	FdU	Topotecan	Irinotecan
Hsa-miR-224	−0.32[1]	nd[2]	nd	nd	nd
Hsa-miR-23a*	0.36	nd[1]	0.35	0.48	0.41
Hsa-miR-24	nd[1]	−0.31	−0.29	−0.32	−0.43
hsa-miR-24-1*	−0.31	nd	nd	nd	nd
hsa-miR-324-3p	0.37	nd	0.34	0.44	0.38
hsa-miR-455-3p	0.36	nd	0.26	nd	nd
hsa-miR-500	0.38	nd	0.37	0.48	0.43
hsa-miR-501-5p	0.37	0.26	0.35	0.48	0.41
hsa-miR-650	0.35	nd	0.38	0.43	0.46
hsa-miR-874	0.36	nd	0.40	0.43	0.49
hsa-miR-1226*	0.37	0.32	0.40	0.44	0.44

[1] Negatively significant correlations are underlined.

[2] "nd" indicates that thecorrelation was less than cutoff (+/− 0.25).

Table 5

Conserved Targets of miR-24[1]

Gene	Aggregate P_{CT}[2]
Top1	0.64
Bcl2-L11	0.98
MTHFR	0.68

[1] http://www.targetscan.org/cgi-bin/targetscan/vert_50/targetscan.cgi?mirg=hsa-miR-24

[2] Scores for preferential conservation of the site