Genome-Wide mRNA and microRNA Profiling of the NCI 60 Cell-Line Screen and Comparison of FdUMP[10] with Fluorouracil, Floxuridine, and Topoisomerase 1 Poisons

William H. Gmeiner¹, William C. Reinhold², and Yves Pommier²

Abstract

A profile of microRNA (miRNA) 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), 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, whereas expression of glycosyltransferases (XYLT2) that use 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 miRNA with a high aggregate PCT score for topoisomerase 1 (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]. Mol Cancer Ther; 9(12); 3105–14. ©2010 AACR.

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 sensitivities are well documented (4–6). Although the general response [e.g., growth inhibitory 50 (GI50) values] profiles across the NCI-60 cell-line screen can indicate mechanistic similarities and differences between two or more drugs, which is the basis of the COMPARE approach (7, 8), additional mechanistic insights can be obtained from analysis of gene expression signatures in concert with microRNA (miRNA) expression signatures (6).

The poly-fluoropyrimidine antitumor agent FdUMP[10] has shown promising activity in preclinical studies, including strong activity toward malignancies (e.g., leukemia; ref. 9) that are not responsive to traditional fluoropyrimidine chemotherapeutics, such as fluorouracil (5FU) and floxuridine (FdU; Fig. 1A). COMPARE analysis of FdUMP[10] across the NCI-60 cell-line screen data revealed similarities between FdUMP[10] and the topoisomerase 1 (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, showing 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 (Fig. 1A) across the NCI-60 cell-line screen. Our results further show mechanistic similarities of FdUMP[10] with Top1 poisons on the basis of expression profile similarities, while clarifying distinct mechanistic features of FdUMP[10] on the basis of the unique mRNA and microRNA profile for this drug, which is distinct from both alternative fluoropyrimidines (e.g., 5FU and FdU) as well as from other Top1 poisons (e.g., irinotecan and topotecan).
Materials and Methods

Cell culture and RNA purifications
All cell lines were obtained directly from the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, 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 miRNA were as described previously (5).

Drug activities
Drug activities were obtained from the Developmental Therapeutics Program.3 They are graphed in Fig. 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, 5, 6, and 7 are Pearson’s, and were calculated in Excel 2008 for MacIntosh. Statistical significance for $N = 60$ is 0.254 at $P < 0.05$.

Transcript probe and probe-set data
For the genes described in Table 2, the transcript expression levels were determined using the probes from five platforms. These probes included, from Affymetrix (Affymetrix Inc.), the ~60,000-feature Human Genome U95 set (HG-U95; ref. 12); the ~44,000-feature Human Genome U133 (HG-U133; ref. 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; ref. 13). From Agilent (Agilent Technologies, Inc.), we used the

---

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, whereas the sensitivity profile for FdUMP[10] indicates similarities with the Top1 poisons topotecan and irinotecan. Top1 is the sole target for topotecan and irinotecan. Gene expression profiling reveals mechanistic similarities of FdUMP[10] with these Top1 poisons.

3 http://dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp
~41,000-feature Whole Human Genome Oligo microarray (14). Normalization of HG-U95 and HG-U133 was done by GC robust multi-array average (GCRMA; ref. 15). Normalization of HG-U133 Plus 2.0 and the Whole Human Genome Oligo microarray was by robust multi-array analysis (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.

Table 1. Drug activities across leukemia and central nervous system cell lines and summary statistics for complete NCI-60 cell-line screen

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

Note: Drug activities are GI50s, presented as −log10 values. Drug activities obtained from http://dtp.nci.nih.gov/dtpstandard/cancerscreeningdata/index.jsp.

Abbreviations: CNS, central nervous system; LE, leukemia; na, not applicable; NSC, National Service Center.

Relative gene expression levels were determined on the basis of 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 log2 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 with 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 and/or 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 ≥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 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 miRNA expression levels

We have described previously the purification, quality assessment, and expression level determinations of the miRNA shown in Table 2 (14). In brief, total RNA (100 ng) was labeled following the recommendations of Agilent Technologies (miRNA Microarray System protocol version 1.5) and hybridized to the Agilent Technologies
Human miRNA microarray (version 2). Scanning and data extraction of the arrays were done as recommended by Agilent Technologies. These expression data are available at CellMiner.6

Results and Discussion

Sensitivity and/or resistance profiles

The relative sensitivity and/or resistance profile for a drug over the 60 cell lines included in the NCI screen provides valuable information that can group drugs on the basis of 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 and/or resistance profile for FdUMP[10] is distinct from that of 5FU, consistent with these two fluoropyrimidine drugs having distinct cytotoxic mechanisms (Fig. 1B). Although the sensitivity and/or resistance profile for FdUMP[10] more closely resembles FdU than 5FU, distinct differences between these two types of fluoropyrimidines are also evident in the sensitivity and/or resistance data and profile (Fig. 1).

The sensitivity profile for FdUMP[10] has several features that distinguish it from other fluoropyrimidine 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 fluoropyrimidine drugs are renal malignancies (Fig. 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 (Tables 1 and 3). Interestingly, the dynamic range of GI50 values for FdUMP[10] was four orders of magnitude, which greatly exceeds the other drugs included in this study (Tables 1 and 3). 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 log10 (with a range of 0.23 to 2.32). The second advantage 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 log10.

The overall sensitivity and/or resistance profile for FdUMP[10] resembles that for the Top1 poisons topotecan and irinotecan nearly as closely as it resembles FdU (Fig. 1B). Further, the sensitivity and/or 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 corre-

<table>
<thead>
<tr>
<th>Gene</th>
<th>FdUMP[10]</th>
<th>5FU</th>
<th>FdU</th>
<th>Topotecan</th>
<th>Irinotecan</th>
</tr>
</thead>
<tbody>
<tr>
<td>TYMS (TS)</td>
<td>nd*</td>
<td>nd</td>
<td>0.26</td>
<td>0.30</td>
<td>0.35</td>
</tr>
<tr>
<td>UMP5</td>
<td>nd</td>
<td>0.37</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>DPYD</td>
<td>nd</td>
<td>−0.34</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>NME1</td>
<td>0.47</td>
<td>0.27</td>
<td>0.35</td>
<td>0.33</td>
<td>0.35</td>
</tr>
<tr>
<td>XLYT2</td>
<td>0.44</td>
<td>nd</td>
<td>nd</td>
<td>0.29</td>
<td>nd</td>
</tr>
<tr>
<td>OGT</td>
<td>−0.26</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Abbreviation: TS, thymidylate synthetase.
*nd, the correlation was less than cutoff (≥0.25).

| Table 2. Correlation with select genes implicated in nucleoside metabolism |
|-----------------------------|-----------|-----|-----|-----------|------------|
| Gene | FdUMP[10] | 5FU | FdU | Topotecan | Irinotecan |
| TYMS (TS)| nd*       | nd  | 0.26| 0.30      | 0.35       |
| UMP5     | nd        | 0.37| nd  | nd        | nd         |
| DPYD     | nd        | −0.34| nd  | nd        | nd         |
| NME1     | 0.47      | 0.27| 0.35| 0.33      | 0.35       |
| XLYT2    | 0.44      | nd  | nd  | 0.29      | nd         |
| OGT      | −0.26     | nd  | nd  | nd        | nd         |

| Abbreviation: TS, thymidylate synthetase. |
*nd, the correlation was less than cutoff (≥0.25). |

Table 3. Comparative potency (GI50 –log10 values) of the five drugs studied across the NCI-60

<table>
<thead>
<tr>
<th>−log10 GI50</th>
<th>FdUMP[10]</th>
<th>FdU</th>
<th>Topotecan</th>
<th>Irinotecan</th>
<th>5FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSC-697912</td>
<td>7.15</td>
<td>4.64</td>
<td>5.83</td>
<td>7.19</td>
<td>4.89</td>
</tr>
<tr>
<td>NSC-27640</td>
<td>4.30</td>
<td>3.31</td>
<td>3.61</td>
<td>5.45</td>
<td>4.08</td>
</tr>
<tr>
<td>NSC-609699</td>
<td>8.30</td>
<td>5.98</td>
<td>6.58</td>
<td>8.07</td>
<td>6.63</td>
</tr>
<tr>
<td>NSC-616348</td>
<td>4.00</td>
<td>2.67</td>
<td>2.96</td>
<td>2.63</td>
<td>2.55</td>
</tr>
<tr>
<td>NSC-19893</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NSC, National Service Center.

6 http://discover.nci.nih.gov/cellminer/
lation coefficients derived from the sensitivity and/or resistance profile data for FdUMP[10], 5FU, FdU, topotecan, and irinotecan is shown in Table 4. SN-38, the active metabolite of irinotecan gave comparable results (data are 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 miRNA 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 Supplementary Table S1, together with a gene ontology analysis identifying pathways correlated with drug sensitivity.

Genes involved in endocytosis

Previous studies have shown that cells deficient in thymidine kinase 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 (\( r = 0.40 \); Fig. 2 and Table 5).

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 subcellular routing of internalized proteins (19). SNF8 tends to have the lowest expression in the breast carcinoma cells (Fig. 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).

Table 4. Correlation between drug activity profiles in the NCI-60

<table>
<thead>
<tr>
<th>Drug</th>
<th>FdUMP[10]</th>
<th>5FU</th>
<th>FdU</th>
<th>Topotecan</th>
<th>Irinotecan</th>
</tr>
</thead>
<tbody>
<tr>
<td>FdUMP[10]</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5FU</td>
<td>0.55</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FdU</td>
<td>0.78</td>
<td>0.59</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Topotecan</td>
<td>0.66</td>
<td>0.35</td>
<td>0.63</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Irinotecan</td>
<td>0.63</td>
<td>0.42</td>
<td>0.66</td>
<td>0.73</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note: Pearson’s correlation coefficient of 0.414 corresponds to a significant correlation (\( P < 0.001 \)) in the absence of multiple comparisons correction. Data obtained from: http://faculty.fortlewis.edu/CHEW_B/Documents/Table%20of%20critical%20values%20for%20Pearson%20correlation.htm).

Table 5. Correlation of selected genes implicated in endocytosis

<table>
<thead>
<tr>
<th>Gene</th>
<th>FdUMP[10]</th>
<th>5FU</th>
<th>FdU</th>
<th>Topotecan</th>
<th>Irinotecan</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLTC</td>
<td>0.41</td>
<td>-0.25</td>
<td>0.29</td>
<td>nd(^a)</td>
<td>nd</td>
</tr>
<tr>
<td>SNF8</td>
<td>0.42</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>ANXA6</td>
<td>0.42</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>APPBP2</td>
<td>0.40</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

\(^a\)nd, the correlation was less than cutoff (±0.25).
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 has been found highly expressed in breast and ovarian cancer (21), Fig. 2 shows that MCF7 cells exhibit very high expression of APPBP2, whereas APPBP2 expression does not seem 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 subcellular 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 fluoropyrimidine drugs, such as 5FU, is that fewer steps of metabolic activation are required to produce FdUMP andFdUTP, the DNA-directed fluoropyrimidine 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 2. Somewhat surprisingly, the TYMS gene encoding thymidylate synthetase (23) was not strongly correlated with FdUMP[10] sensitivity, but was for irinotecan ($r = 0.35$), topotecan ($r = 0.30$), and FdU ($r = 0.26$). Thymidylate synthetase expression also did not correlate with 5FU sensitivity. The lack of correlation of FdUMP[10] sensitivity with thymidylate synthetase expression likely results from the high efficiency of thymidylate inhibition with FdUMP[10] treatment (24), whereas the correlation of thymidylate synthetase expression with Top1 poisons may reflect a correlation with an elevated proliferation rate. Uridine monophosphate synthetase (UMPS; ref. 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. Although thymidine kinase deficiency results in resistance to FdU, but not FdUMP[10] (17), the lack of correlation with FdU sensitivity probably indicates a

### Table 6. MicroRNA correlations

<table>
<thead>
<tr>
<th>MicroRNA</th>
<th>FdUMP[10]</th>
<th>5FU</th>
<th>FdU</th>
<th>Topotecan</th>
<th>Irinotecan</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-224</td>
<td>-0.32a</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>hsa-miR-23a*</td>
<td>0.36</td>
<td>nd</td>
<td>0.35</td>
<td>0.48</td>
<td>0.41</td>
</tr>
<tr>
<td>hsa-miR-24</td>
<td>nd</td>
<td>-0.31a</td>
<td>-0.29a</td>
<td>-0.32a</td>
<td>-0.43a</td>
</tr>
<tr>
<td>hsa-miR-24-1*</td>
<td>-0.31a</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>hsa-miR-324-3p</td>
<td>0.37</td>
<td>nd</td>
<td>0.34</td>
<td>0.44</td>
<td>0.38</td>
</tr>
<tr>
<td>hsa-miR-455-3p</td>
<td>0.36</td>
<td>nd</td>
<td>0.26</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>hsa-miR-500</td>
<td>0.38</td>
<td>nd</td>
<td>0.37</td>
<td>0.48</td>
<td>0.43</td>
</tr>
<tr>
<td>hsa-miR-501-5p</td>
<td>0.37</td>
<td>0.26</td>
<td>0.35</td>
<td>0.48</td>
<td>0.41</td>
</tr>
<tr>
<td>hsa-miR-650</td>
<td>0.35</td>
<td>nd</td>
<td>0.38</td>
<td>0.43</td>
<td>0.46</td>
</tr>
<tr>
<td>hsa-miR-874</td>
<td>0.36</td>
<td>nd</td>
<td>0.40</td>
<td>0.43</td>
<td>0.49</td>
</tr>
<tr>
<td>hsa-miR-1226*</td>
<td>0.37</td>
<td>0.32</td>
<td>0.40</td>
<td>0.44</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*aNegatively significant correlations.
bnd, the correlation was less than cutoff ($r < 0.25$).
moderate range in DTYMK expression across the 60 cell lines with relatively few cell lines highly deficient in DTYMK expression.

The NME1 gene (28) that encodes nucleoside diphosphate kinase A correlated with all five drugs, with the strongest correlation for FdUMP (r = 0.47) and the weakest correlation for 5FU (r = 0.27; Table 2). NME1 is also known as the metastasis suppressor NM23 (29). NME1 expression seems to vary widely across cell lines irrespective of their tissue of origin (Fig. 2).

Figure 2. Gene expression profiles across the NCI-60 for several genes significantly correlated with FdUMP (see Tables 2 and 5 for further details).

http://www.genecards.org/cgi-bin/carddisp.pl?gene=NME1&search=nme1
Xylosyltransferase 2 (XYLT2; ref. 30), a glycosyltransferase that transfers xylose from UDP sugars to serine residues of proteins, correlated with sensitivity to FdUMP[10] (r = 0.44; Table 2). XYLT2 seems consistently low in leukemias (Fig. 2) and did not significantly correlate with sensitivity to either FdU or 5FU (Table 2). Interestingly, O-linked N-acetyl glucosamine (GlcNAc) transferase (OGT; ref. 31) negatively correlated with FdUMP[10] sensitivity (r = −0.26), and has overall high expression in the leukemias (Fig. 2), but did not correlate with either 5FU or FdU (Table 2). 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 shown 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 and/or resistance profile for FdUMP[10] is more highly correlated with Top1 poisons than with 5FU (Fig. 1B and Table 4). Sensitivity to topotecan and irinotecan correlates only weakly with TOP1 expression (data are not shown; refs. 32–34). The weak correlation of topotecan and irinotecan with TOP1 expression likely results from regulation by miR-24 (Table 6). Elevated miR-24 potentially negatively correlates with sensitivity to FdUMP[10] across the NCI-60 cell-line panel. Two miRNAs (miR-224 and miR-24-1*) had significant negative correlation with FdUMP[10] sensitivity (Table 6). Interestingly, Top1 is likely a target for regulation by miR-24-1*, as playing an important role in cellular sensitization to FdUMP[10].

**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 (TGFB3 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 that downregulation of prosurvival proteins sensitizes cells to FdUMP[10]. The gene class that displayed the greatest overrepresentation among genes whose downregulation 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, ZNF266, and ZNF649); twice as many as were identified among those genes whose upregulation 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].

**DNA damage and repair**

Treatment of cancer cells with FdUMP[10] results in substantial DNA damage with DNA double-strand breaks (DSB) initially evident approximately 16 hours following drug treatment (Gmeiner W.H. 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 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.

**miRNA correlations**

The role of miRNA in regulating gene expression and its significance for cancer progression and treatment is being increasingly recognized (6). We did a miRNA profile establishing to what extent miRNA up- or down-regulation was associated with cellular sensitivity to FdUMP[10] across the NCI-60 cell-line panel. Two miRNAs (miR-224 and miR-24-1*) had significant negative correlation with FdUMP[10] sensitivity (Table 6). 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 9q 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 2). Interestingly, Top1 is likely a target for regulation by miR-24 (Table 6). Elevated miR-24 potentially

---

**Note:**

http://www.mirbase.org/cgi-bin/mirna_entry.pl?acc=MI0000800
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 (BCl2t1 transcript variants 1, 6, 7, and 8), p53 inducible nuclear protein (TP53INP1), Ras p21 protein activator (RasA1), p27, and O-linked N-acetylglucosamine (GlcNAc) transferase. Among all the miRNAs 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 and SMAD5) and XLYT1 (TargetsCan). FdUMP[10] sensitivity also has significant positive correlation with expression of 29 miRNAs ($r > 0.25$). A summary of the highest positive miRNA correlations for FdUMP[10] is included in Table 6.

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 fluoropyrimidines 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 (Table 4), 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 $7.1 \times 10^{-8}$ mol/L, which is 324 fold more potent than 5FU (average GI50 = $2.3 \times 10^{-5}$ mol/L). 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 reinforce 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 whereas both FdUMP[10] and FdU are DNA-directed fluoropyrimidines 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) 5FU is not a DNA-directed fluoropyrimidine (39). In contrast, FdUMP[10] is likely 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 shows 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 miRNA 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.

Disclosure of Potential Conflicts of Interest

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

Grant Support

This work was supported by NIH-NCI CA102532 (W.H. Gmeiner) and by the NIH Intramural Program, National Cancer Institute, Center for Cancer Research (Z01 BC 006161–17LMP).

Received 07/16/2010; revised 09/21/2010; accepted 09/27/2010; published 12/14/2010.

References


Genome-Wide mRNA and microRNA Profiling of the NCI 60 Cell-Line Screen and Comparison of FdUMP[10] with Fluorouracil, Fluorouridine, and Topoisomerase 1 Poisons

William H. Gmeiner, William C. Reinhold and Yves Pommier