

Identification of 5-fluorouracil-inducible Target Genes Using cDNA Microarray Profiling¹

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ABSTRACT

The fluoropyrimidine 5-Fluorouracil (5-FU) is widely used in the treatment of cancer. To identify novel downstream mediators of tumor cell response to 5-FU, we used DNA microarray technology to identify genes that are transcriptionally activated by 5-FU treatment in the MCF-7 breast cancer cell line. Of 2400 genes analyzed, 619 were up-regulated by >3-fold. Highly up-regulated genes (>6-fold) with signal intensities of >3000 were analyzed by Northern blot. Genes that were consistently found to be up-regulated were spermine/spermidine acetyl transferase (SSAT), annexin II, thymosin- β -10, chaperonin-10, and MAT-8. Treatment of MCF-7 cells with the antifolate tomudex and DNA-damaging agent oxaliplatin also resulted in up-regulation of each of these targets. The 5-FU-induced activation of MAT-8, thymosin- β -10, and chaperonin-10 was abrogated by inactivation of p53 in MCF-7 cells, whereas induction of SSAT and annexin II was significantly reduced in the absence of p53. Moreover, each of these genes contained more than one potential p53-binding site, suggesting that p53 may play an important regulatory role in 5-FU-induced expression of these genes. In addition, we found that basal expression levels of SSAT, annexin II, thymosin β -10, and chaperonin-10 were increased (by ~2–3-fold), and MAT-8 expression dramatically increased (by ~10-fold) in a 5-FU-resistant colorectal cancer cell line (H630-R10) compared with the parental H630 cell line, suggesting these genes may be useful biomarkers of resistance. These results demonstrate the potential of DNA microarrays to identify novel genes involved in mediating the response of tumor cells to chemotherapy.

INTRODUCTION

The fluoropyrimidine drug 5-FU⁴ is used in the treatment of gastrointestinal, breast and head and neck cancers. 5-FU is converted intracellularly to fluorodeoxyuridine monophosphate, which, together with 5,10-methylene tetrahydrofolate, forms a stable ternary complex with TS, resulting in enzyme inhibition. TS catalyzes the reductive methylation of dUMP by 5,10-methylene tetrahydrofolate to produce dTMP and dihydrofolate (1). As this reaction provides the sole *de novo* intracellular source of dTMP, which is essential for DNA replication and repair, TS inhibition results in DNA damage. Non-TS-directed mechanisms of cytotoxicity have also been described for 5-FU, such as misincorporation of fluoronucleotides, into DNA and RNA (1). The major limitation to the clinical use of 5-FU is acquired or inherent resistance. *In vitro* and *in vivo* studies have demonstrated that increased TS expression correlates with increased resistance to 5-FU (2). Other upstream determinants of 5-FU chemosensitivity

include the 5-FU-degrading enzyme dihydropyrimidine dehydrogenase and 5-FU-anabolic enzymes, such as orotate phosphoribosyl transferase (1). However, it is likely that events downstream of TS inhibition, such as activation of DNA damage response pathways, also play key roles in determining the cellular response to 5-FU. Identification of such signaling pathways would greatly facilitate the development of new therapeutic strategies to improve the efficacy of 5-FU-based chemotherapy. In this study, we have used DNA microarray technology to investigate changes in the transcriptional profile of the MCF-7 breast cancer cell line after treatment with 5-FU. Our analysis has identified transcriptional target genes that are induced not only by 5-FU but also by the TS-specific antifolate TDx and DNA-damaging agent oxaliplatin and which may therefore be important downstream mediators of tumor cell response to chemotherapy.

MATERIALS AND METHODS

Tissue Culture. MCF-7 breast cancer and H630 and H630-R10 colon cancer cell lines were maintained in DMEM supplemented with 10% dialyzed FCS, 1 mM sodium pyruvate, 2 mM L-glutamine, and 50 μ g/ml penicillin/streptomycin (all from Life Technologies, Inc., Paisley, Scotland). M7TS90 cells (3) were maintained in MCF-7 medium supplemented with 100 μ g/ml G418 (Life Technologies, Inc.), 1 μ g/ml puromycin, and 1 μ g/ml tetracycline (both from Sigma, Poole, Dorset, England). M7TS90-E6 cells (3) were maintained in M7TS90 medium supplemented with 200 μ g/ml hygromycin (Life Technologies, Inc.). All cell lines were grown in 5% CO₂ at 37°C.

Microarray Hybridization, Detection, and Scanning. RNA was collected from untreated MCF-7 cells (control) or after treatment with 10 μ M 5-FU for 6, 12, 24, and 48 h. Ten micrograms of RNA from each time point were combined for both the control and 5-FU-treated samples. Labeled cDNA probes were prepared from 2- μ g aliquots of each pooled RNA sample. cDNA synthesized from control cells was labeled with biotin, and cDNA synthesized from 5-FU-treated samples was labeled with DNP. Labeled probes were purified by ethanol precipitation, and membrane-based chemiluminescence analysis was carried out to determine labeling efficiency. The Micromax Human cDNA Array (NEN Lifesciences, Boston, MA) containing 2400 genes was used in this study. The biotin- and DNP-labeled cDNA probes were combined and hybridized to the microarray for 16 h in a humid incubator at 65°C. The microarray was washed in 0.5 \times SSC and 0.01% SDS for 5 min at room temperature with gentle agitation, followed by a 5-min wash in 0.06 \times SSC and 0.01% SDS and a 2-min wash in 0.06 \times SSC. Hybridized cDNA probes were detected using the tyramide signal amplification detection system according to the manufacturer's instructions (NEN Lifesciences). Biotin-labeled cDNA (derived from untreated cells) was visualized using the Cy5 reporter, and DNP-labeled cDNA (derived from 5-FU-treated cells) was detected using the Cy3 reporter. Scanning of the microarray was performed by NEN Lifesciences using a ScanArray confocal laser scanner (GSI Lumonics, Inc.). The intensity of each hybridized cDNA was evaluated using ImaGene analysis software (BioDiscovery, Inc.), and the Cy3: Cy5 ratio for each gene was calculated.

Northern Blot Analysis. Northern blots were performed as described previously (3). DNA probes for Northern blotting were generated by PCR using cDNA derived from 1 μ g of MCF-7 total RNA as a template. The primer sequences are as follows: SSAT: Forward, 5'-GCT AAA TTC GTG ATC CGC-3'; Reverse, 5'-CAA TGC TGT GTC CTT CCG-3'; Annexin II: Forward, 5'-GGG TGA TCA CTC TAC ACC-3'; Reverse, 5'-CAG TGC TGA TGC AGG TTC-3'; Thymosin β -10: Forward, 5'-TCG GAA CGA GAC TGC

Received 12/30/02; accepted 6/2/03.

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¹ Supported by Cancer Research UK, Ulster Cancer Foundation, and The Research and Development Office, Northern Ireland.

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⁴ The abbreviations used are: 5-FU, 5-fluorouracil; TS, thymidylate synthase; TDx, tomudex; DNP, dinitrophenol; Cy, cyanine; hsp, heat shock protein; SSAT, spermine/spermidine acetyl transferase.

ACG-3'; Reverse, 5'-CTC TTC CTC CAC ATC ACG-3'; MAT-8: Forward, 5'-GCT CTG ACA TGC AGA AGG-3'; Reverse, 5'-CCT CCA CCC AAT TTC AGC-3'; Chaperonin-10: Forward, 5'-GTA ATG GCA GGA CAA GCG-3'; Reverse, 5'-GGG CAG CAT GTT GAT GC-3'; 18S: Forward 5'-CAG TGA AAC TGC GAA TGG-3'; Reverse 5'-CCA AGA TCC AAC TAC GAG-3'.

Western Blot Analysis. Thirty micrograms of protein were resolved by SDS-polyacrylamide gel (12%) as described previously (3). The gels were electroblotted onto Hybond membranes (Hybond-P; Amersham). Antibody staining was performed with a chemiluminescence detection system (Super-signal; Pierce) using the p53 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in conjunction with horseradish peroxidase-conjugated sheep antimouse secondary antibody. Equal lane loading was assessed using a mouse monoclonal glyceraldehyde-3-phosphate dehydrogenase antibody (Biogenesis, Poole, United Kingdom).

RESULTS

DNA Microarray Analysis of Gene Expression after Treatment with 5-FU. To identify novel markers of sensitivity or resistance to 5-FU, we carried out cDNA-based microarray analysis after treatment of MCF-7 breast cancer cells with 10 μ M 5-FU (corresponding to an \sim IC₆₀ dose at 72 h). RNA derived from untreated and 5-FU-treated MCF-7 cells was reverse transcribed, labeled, and hybridized to a 2400 gene cDNA microarray. Bound cDNA was detected using Cy3 (5-FU treated) or Cy5 (control) reporter dyes. The expression profile in the treated and untreated populations was compared and expressed as a Cy3:Cy5 ratio. We found that 619 genes (>25% of genes analyzed) were up-regulated by >3-fold. In contrast, only 16 genes were down-regulated by >3-fold, indicating that 5-FU treatment resulted in widespread transcriptional activation. Potential target genes were initially grouped according to their function using the DRAGON database (Database Referencing of Array Genes Online).⁵ The biological functions of the genes identified by the microarray analysis were diverse and include cell cycle regulators; structural, ribosomal, apoptotic, and mitochondrial genes; as well as genes involved in signal transduction pathways and polyamine metabolism (Table 1). The manufacturer of the DNA microarray defined changes in gene expression of >3-fold as biologically significant. Our data set was obtained from samples pooled from several time points and represents the cumulative increase in gene expression between 6 and 48 h after treatment with 5-FU. Therefore, we chose a higher cutoff of >6-fold induction when selecting genes for further investigation. Genes were also selected on the basis of their signal intensities, with intensities of >3000 considered to be sufficiently high compared with background.

Northern Blot Analysis of Gene Expression after Treatment with 5-FU. Novel genes that were consistently found to be up-regulated after treatment with 5-FU by Northern blot analysis were SSAT, annexin II, thymosin- β -10, chaperonin-10, and MAT-8 (Fig. 1A). SSAT catalyzes the rate-limiting step in the catabolism of the polyamines spermine and spermidine (4). SSAT mRNA was induced 15-fold compared with control 48 h after treatment with 10 μ M 5-FU, and this induction was maintained at 72 h (Fig. 1A). Annexin II has been reported to regulate cell proliferation and apoptosis (5). Induction of annexin II mRNA in response to 5-FU followed a similar pattern to that observed for SSAT with levels \sim 5-fold higher than control at 72 h (Fig. 1A). Thymosin- β -10 has also been reported to contribute to the regulation of apoptosis (6). We found that thymosin- β -10 was up-regulated 72 h after treatment with 5-FU with levels 8-fold above control (Fig. 1A). MAT-8 is a transmembrane protein that regulates chloride ion transport (7). We found that MAT-8 ex-

Table 1 Functional grouping of genes identified by cDNA microarray analysis as being up-regulated by 5-FU in MCF-7 cells

Potential target genes were grouped according to their function using the DRAGON database.

Family	Examples	Fold induction	Signal intensity
Signal transduction	Raf	3.9	8686
	K-ras	4.8	9662
	SLAP	5.0	5391
Apoptosis	Phosphoinositide 3-kinase	3.2	918
	COP9 homolog (HCOP9)	8.6	1587
	Apoptosis specific protein	4.6	1625
	APO-1 cell surface antigen	4.2	4453
Cell cycle	FLIP protein	3.7	5793
	Cyclin G	8.5	13789
	CDC2	3.1	1779
Structural	Cyclin-dependent protein kinase-2	5.9	3416
	Thymosin β -10	8.5	27041
	Myosin light chain	3.2	397
Polyamine metabolism	Gelsolin	7.3	18482
	Thymosin β -4	4.3	46355
	SSAT	13.0	3662
Cell surface	Spermidine synthase	3.7	3874
	Spermidine aminopropyltransferase	5.0	6633
	MAT-8 protein	10.1	6522
Mitochondrial	Annexin II	12.3	24463
	Annexin IV	9.3	4101
	FGF ^a receptor 2	4.9	684
	Transmembrane 4 superfamily protein	3.2	491
	Chaperonin 10	11.6	8478
	Enoyl-CoA hydratase	3.4	2512
Ribosomal proteins	Nicotinamide nucleotide transhydrogenase	4.7	1508
	Ribosomal protein S28	10.9	24039
	Ribosomal protein L37	3.0	723
	L23 mRNA for putative ribosomal protein	4.6	12662
	Ribosomal protein L7	5.5	1724

^a FGF, fibroblast growth factor.

pression was up-regulated 24 h after 5-FU treatment and continued to increase throughout the time course to levels that were 11-fold higher than control by 72 h (Fig. 1A). Chaperonin-10 is a mitochondrial hsp (8). Chaperonin-10 was up-regulated 72-h post-treatment with 5-FU with levels 4-fold higher than control (Fig. 1A).

Northern Blot Analysis of Target Gene Expression after Treatment with TDX and Oxaliplatin. Recently, specific folate-based inhibitors of TS have been developed, of which TDX is the first to be approved for clinical use (9). The platinum-based, DNA-damaging agent oxaliplatin has demonstrated synergistic activity with TS inhibitors in preclinical studies (10) and is used in the treatment of advanced colorectal cancer (11). We examined the expression of the 5-FU-inducible target genes after treatment of MCF-7 cells with \sim IC₆₀s of TDX (10 nM) and oxaliplatin (10 μ M) for 72 h (Fig. 1B). SSAT mRNA was up-regulated 15-fold in response to treatment with TDX and 6-fold in response to oxaliplatin (Fig. 1B). Annexin II mRNA was also up-regulated (by \sim 5-fold) in response to TDX and oxaliplatin. Expression of thymosin- β -10 mRNA was up-regulated \sim 5-fold in response to TDX and \sim 6-fold in response to oxaliplatin (Fig. 1B). MAT-8 mRNA expression was also induced in response to TDX and oxaliplatin by \sim 8-fold in each case (Fig. 1B). Treatment with TDX caused a moderate 1.5-fold induction of chaperonin-10, and oxaliplatin treatment resulted in \sim 2.5-fold induction of this gene (Fig. 1B). Thus, the 5-FU target genes identified by the cDNA microarray screen were also found to be induced by TDX and oxaliplatin.

Effect of p53 Inactivation on Target Gene Induction. p53 has been reported previously to play an important role in downstream signaling after 5-FU treatment (3). To determine whether p53 might play a role in 5-FU-mediated target gene up-regulation, we examined the sequences of the 5-FU-inducible genes for regions of homology to

⁵ Internet address: <http://pevsnerlab.kennedykrieger.org/dragon.htm>.

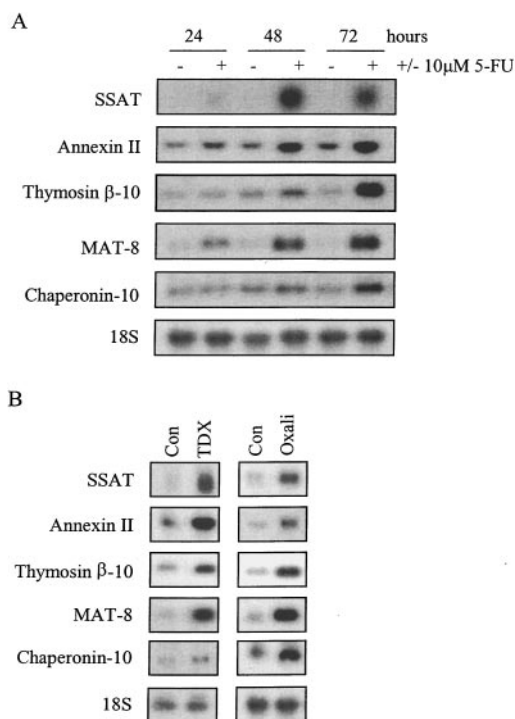


Fig. 1. *A*, Northern blot analysis of SSAT, annexin II, thymosin β -10, MAT-8, and chaperonin-10 mRNA expression in MCF-7 cells treated for 24, 48, and 72 h with no drug (-) or 10 μ M 5-FU (+). 18S rRNA expression was assessed as a loading control. *B*, Northern blot analysis of SSAT, annexin II, thymosin β -10, MAT-8, and chaperonin-10 mRNA expression in MCF-7 cells treated for 72 h with no drug (Con), 10 nM TDX (TDX), or 10 μ M oxaliplatin (Oxali). 18S rRNA expression was assessed as a loading control.

putative p53-binding sites using the TRANSFAC database (12).⁶ We found that the *SSAT* and *MAT-8* genes each contained three putative p53-binding sites with >85% homology, and the *annexin II* and *thymosin- β -10* genes each contained two sites. The *chaperonin-10* and *hsp60* genes are transcribed from the same promoter, and this locus contained 16 putative p53-binding sites. This suggested that p53 might play a role in the regulation of expression of these genes. We therefore compared expression of each of the 5-FU-inducible genes in p53 wild-type (M7TS90) and p53-null (M7TS90-E6) isogenic cell lines, derived from MCF-7 cells as described previously (3). In the M7TS90 cell line, SSAT mRNA expression was induced after treatment with 5-FU for 72 h to a similar extent as in the parental MCF-7 line (~13-fold), whereas expression in the p53-null M7TS90-E6 cell line was only up-regulated by ~2-fold (Fig. 2A). Induction of annexin II mRNA was also reduced in the p53-null cell line (2-fold with respect to control) compared with the p53 wild-type line (7-fold with respect to control; Fig. 2A). In M7TS90 cells, MAT-8, thymosin- β -10 and chaperonin-10 mRNAs were each induced by 5-FU treatment by between 8- and 10-fold (Fig. 2A). In contrast, expression of these genes was unaltered by 5-FU treatment in the p53-null M7TS90-E6 cell line (Fig. 2A). These results suggested an important regulatory role for p53 in up-regulating each of these target genes; therefore, we also examined the effect of 5-FU, TDX, and oxaliplatin on p53 protein expression. MCF-7 cells were exposed to ~IC₆₀s of each agent for 48 h (Fig. 2B). p53 protein levels were up-regulated after exposure to 10 μ M 5-FU (7-fold), TDX (3-fold), and oxaliplatin (8-fold; Fig. 2B). Collectively, these results suggested a key transcriptional regulatory role for p53 in the response to 5-FU, TDX, and oxaliplatin in this cell line.

⁶ Internet address: <http://transfac.gbf.de/TRANSFAC>.

Expression of Target Genes in the 5-FU-resistant H630-R10 Cell Line. We next examined the expression of the validated target genes in H630 colon cancer cells after exposure to 5-FU (Fig. 3). We discovered that expression of SSAT and MAT-8 mRNA in H630 cells was induced by ~5–6-fold after treatment with 10 μ M 5-FU. Chaperonin-10 mRNA expression was also up-regulated by ~3-fold in response to 5-FU; however, the expression of annexin II and thymosin- β -10 mRNA was only marginally up-regulated (by ~2-fold) after exposure to 10 μ M 5-FU (Fig. 3). We also compared basal expression of the 5-FU-inducible genes in the H630 colorectal cancer cell line and a 5-FU-resistant daughter line, H630-R10. We found that expression of MAT-8 mRNA was dramatically increased in the 5-FU-resistant H630-R10 cell line compared with the parental H630 cell line (by ~10-fold; Fig. 3). Expression of SSAT, annexin II, and thymosin- β -10 mRNAs was also elevated in the resistant cell line (by ~2-fold in each case), whereas chaperonin-10 expression levels were ~3-fold higher in H630-R10 cells compared with H630 cells (Fig. 3). Thus, the development of 5-FU resistance in H630-R10 cells correlated with increased basal expression of each of the target genes.

DISCUSSION

The assessment of gene expression profiles by cDNA microarray after treatment with chemotherapeutic agents has the potential to

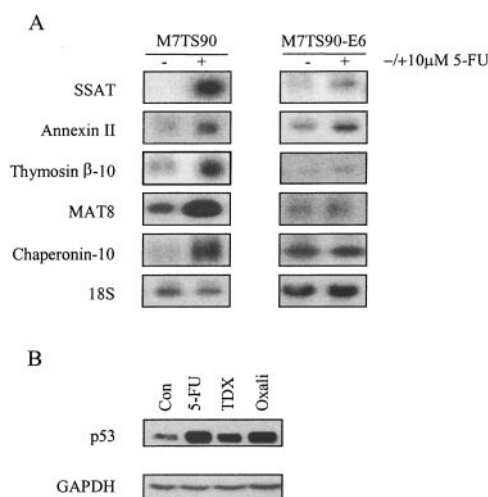


Fig. 2. *A*, Northern blot analysis of SSAT, annexin II, thymosin β -10, MAT-8, and chaperonin-10 mRNA expression in p53 wild-type M7TS90 cells and p53-null M7TS90-E6 cells treated for 72 h with no drug (-) or 10 μ M 5-FU (+). 18S rRNA was assessed as a loading control. *B*, Western blot analysis of p53 expression in MCF-7 cells treated for 72 h with no drug (Con) or IC₆₀s of 5-FU, TDX, or oxaliplatin (Oxali). Glyceraldehyde-3-phosphate dehydrogenase expression was assessed as a loading control.

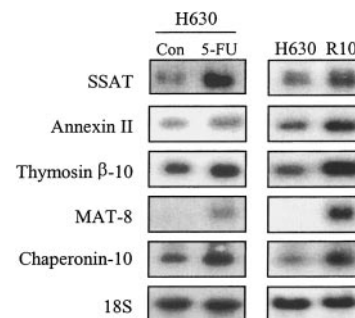


Fig. 3. Northern blot analysis of SSAT, annexin II, thymosin β -10, MAT-8, and chaperonin-10 mRNA expression in H630 cells treated for 72 h with no drug (Con) or 10 μ M 5-FU. Basal expression of these genes was also compared in the H630 cell line and 5-FU-resistant H630-R10 daughter line. For each Northern blot, 18S rRNA expression was used as a loading control.

identify novel signaling pathways involved in mediating the downstream response to these therapies and could greatly facilitate the discovery of novel potential therapeutic targets and/or markers of chemoresistance. Studies examining the transcriptional expression profiles of cancer cell lines and tumors have begun to identify genes that may be associated with response or resistance to these anticancer agents (13–17). In the present study, we have used such an approach to identify genes that are up-regulated after treatment with 5-FU in MCF-7 breast cancer cells. Of 2400 genes analyzed, we found that 619 genes (>25%) were up-regulated by >3-fold, highlighting the widespread up-regulation of gene expression caused by 5-FU treatment. To initially characterize the genes that were transcriptionally activated by 5-FU, we grouped them according to function using the DRAGON database (Table 1). We identified several families of up-regulated genes, including genes encoding structural, mitochondrial, ribosomal, and cell surface proteins, and genes involved in the regulation of cell cycle, apoptosis, and polyamine metabolism. The expression of a number of genes implicated in signal transduction pathways was also up-regulated in response to 5-FU.

The manufacturer of the cDNA microarray recommended that >3-fold induction could be considered biologically significant. However, our data set was generated using RNA samples collected at several time points after 5-FU treatment. As these samples were pooled before analysis, our data set represents the cumulative changes in gene expression between 6- and 48-h post-drug treatment. We therefore used a cut-off of >6-fold induction when selecting genes for validation and further characterization. We also used a signal intensity cutoff of >3000 to ensure identification of genes with signals of sufficient intensity to minimize the effects of background noise. We confirmed that SSAT, annexin II, thymosin- β -10, MAT-8, and chaperonin-10 were consistently up-regulated after treatment with an IC_{60} dose of 5-FU in MCF-7 cells. SSAT causes a reduction in intracellular polyamine levels, which is associated with the induction of apoptosis (4). Annexin II is a member of the annexin family of genes and has been implicated in numerous roles, including the regulation of DNA synthesis, cell proliferation, and apoptosis (5). The G-actin-binding protein thymosin- β -10 is a member of the β -thymosin family of proteins (18) and plays a role in the regulation of apoptosis (6). MAT-8 is a member of the FXDY family of proteins (19) that regulates chloride ion transport across the cell membrane (7). The hsp chaperonin-10 (hsp10) binds hsp60 to regulate folding of mitochondrial proteins (8). To our knowledge, none of these genes have been identified previously as 5-FU-inducible target genes.

We found that $\sim IC_{60}$ s of the TS-targeted antifolate TDX and DNA-damaging agent oxaliplatin also caused up-regulation of each of the target genes. Each of these genes was found to contain potential p53-responsive elements. Importantly, inactivation of p53 in an MCF-7-derived cell line (M7TS90-E6) resulted in significantly reduced levels of 5-FU-mediated induction of SSAT and annexin II mRNA, whereas expression of thymosin- β -10, MAT-8, and chaperonin-10 was not induced in the p53-null setting. These results suggest that p53 may play a role in regulating expression of the target genes in response to 5-FU. In addition, p53 protein was induced in MCF-7 cells treated with $\sim IC_{60}$ doses of 5-FU, TDX, and oxaliplatin. Thus, these agents induced target gene expression and also caused up-regulation of p53, providing additional evidence for the involvement of p53 in regulating these genes.

We also examined expression of the validated target genes in the H630 colorectal cancer cell line and the paired 5-FU-resistant daughter cell line, H630-R10 (2). TS is overexpressed in the H630-R10 cell line by 33-fold compared with the parental line. We found that expression of all five target genes was up-regulated in response to 5-FU in the H630 parent cell line. Interestingly, we also found that

basal expression of all five target genes, in particular MAT-8, was higher in the 5-FU-resistant H630-R10 daughter cell line. This may arise because of the dysregulation of target gene expression in the 5-FU-resistant cell line, because elevated basal expression of these genes was not associated with increased cell cycle arrest or apoptosis. Thus, H630-R10 cells may tolerate higher basal levels of the target genes, suggesting they may be potential biomarkers of resistance.

A key concern with the use of cDNA microarray analysis in relation to cancer therapy is that the evaluation of a large number of genes may identify such a sizeable number of potential target genes that it would be unfeasible to try to confirm the involvement of each of these genes in resistance/response to therapy. Nonetheless, the present study and others have shown that microarray analysis is a powerful technology for the identification of novel genes associated with response or resistance to chemotherapeutic agents (13, 15, 16). However, we were unable to validate the 5-FU-mediated induction of several genes identified by the microarray using Northern blotting, including gel-solin and cyclin G, indicating the importance of target gene validation. Furthermore, Wang *et al.* (20) identified *YES1* from an array study as a gene whose expression was consistently up-regulated in a panel of cell lines resistant to TS inhibitors. However, *YES1* was found to have no direct role in the drug resistance process, and its elevated expression was found to occur as a consequence of its chromosomal location adjacent to *TS*. This study highlights the importance of careful interpretation of validated targets.

In conclusion, using DNA microarray technology, we have identified five novel 5-FU-inducible transcriptional targets: (a) SSAT; (b) annexin II; (c) MAT-8; (d) thymosin β -10; and (e) chaperonin-10. These genes were also up-regulated by TDX and oxaliplatin. Each of these genes contains putative p53-response elements, and 5-FU-mediated induction of these genes was significantly reduced in a p53-null MCF-7 daughter line, suggesting a role for p53 in their regulation. Finally, basal expression of these genes (in particular MAT-8) was higher in a 5-FU-resistant cell line, suggesting that these genes may be potential biomarkers of 5-FU resistance. These results demonstrate the potential of DNA microarrays to identify novel genes involved in mediating the response of tumor cells to chemotherapy.

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