p53-regulated Transcriptional Program Associated with Genotoxic Stress-induced Apoptosis.

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Abstract
Using a genome-wide approach, we sought the identification of p53-regulated genes involved in cellular apoptosis. To this end, we assessed the transcriptional response of HCT116 colorectal cancer cells during apoptosis induced by anticancer drug 5-Fluorouracil (5-FU) as the function of p53 status and identified 230 potential targets that are regulated by p53. Previously identified p53 targets known to be involved in growth arrest and apoptosis were observed to be induced thus validating the approach. Strikingly, we found that p53 regulates gene expression primarily through transcriptional repression (n=189) rather than activation (n=41) and selective blockade of p53-dependent gene repression resulted in the reduction in 5-FU induced apoptosis. Reporter and chromatin-immunoprecipitation (ChIP) assays demonstrated that p53 can suppress the promoter activities of three further studied candidate genes PLK, PTTG1 and CHEK1 but would only bind directly to PTTG1 and CHEK1 promoters, revealing that p53 can repress gene expression through both direct and indirect mechanisms. Moreover, RNAi-mediated knockdown of PLK and PTTG1 expression were sufficient to induce apoptosis, suggesting that repression of novel anti-apoptotic genes by p53 might contribute to a significant portion of the p53-dependent apoptosis. Our data supports the divergent functions of p53 in regulating gene expression that play both synergistic and pleiotropic roles in p53-associated apoptosis.
Introduction:

p53 protein is the most commonly mutated tumor suppressor in human cancers. p53 exerts its function through cell cycle arrest, which allows time for DNA damage repair, or apoptosis, which eliminates cells with damaged DNA (1). It is generally believed that these functions of p53 are mediated by transactivation of p53 through its ability to bind to cis-acting DNA elements within the regulatory regions (2,3). In addition to its ability in transcriptional activation, p53 can also negatively regulate the expression of genes through unknown mechanisms (4,5).

In response to DNA damage and other forms of cellular stress, the levels of p53 protein are greatly increased, and the ability of p53 to bind specific DNA sequences is activated (3). p53 protein levels are regulated post-transcriptionally; thus, the accumulation of p53 following DNA damage results primarily from an increase in protein stability (6). It has become clear that the transcriptional activity of p53 is required for p53-dependent cell cycle arrest; however, the mechanisms by which p53 induces apoptosis are not fully understood (1,3). In particular, it remains unclear as to whether p53-mediated transactivation contributes fully to p53-dependent apoptosis. Unlike the cell cycle inhibitory capacity of p53, which is mediated primarily by p21, a number of genes known to be involved in apoptosis, including BAX, PUMA, FAS/APO-1, NOXA, PIGs, p53AIP1 have been identified as direct transcriptional targets regulated by p53 (3,7).

Although it is widely believed that p53 induces apoptosis through transcriptional activation of its putative apoptotic targets (1,8,9), there is also evidence suggesting that p53-mediated apoptosis and transactivation are uncoupled (10-12), and that p53 binding to its target genes and subsequent induction of apoptotic target genes are not correlated with the apoptotic phenotype (11). Moreover, there is indirect evidence indicating that p53 induces apoptosis through transcriptional repression of anti-apoptotic signals (12-14). Moreover, p53 has been
shown to induce apoptosis in response to one stimulus but growth arrest to another, probably due
to selective transcription of subsets of p53 targets (15,16). Therefore, how p53 modulates
apoptosis remains elusive, and whether transcriptional activation and repression are both
required for p53-induced apoptosis is unclear.

Several studies have been performed to identify p53 target genes on a large scale using
microarray technology (17-22). However, most of these studies focused on the p53
transactivated genes and the whole transcriptional program regulated by p53 in relation to
apoptosis has not been dissected in detail. In addition, previous studies carried out in artificial
systems produces exaggerated levels of overexpressed p53 which, in the case of myc, had been
shown to induce a different set of genes than physiologic levels of myc (23). We sought to
identify the transcriptional programs involved in p53-induced apoptosis and to understand the
regulation of p53 target genes in biologically relevant conditions. To these ends, we took the
advantage of colorectal cancer HCT116 cells which are known to undergo p53-dependent
apoptosis in response to anticancer drug 5-FU, an antimetabolite that activates p53 (24). Thus, by
comparing the temporal gene expression response profiles between HCT116 cells and the p53-
deficient counterparts following 5-FU treatment, we are able to identify 5-FU-induced changes
in gene expression that are either dependent on p53 in general or specific to the drug treatment.
We dissected the p53-dependent transcriptional program in detail and identified approximately
230 genes that are tightly regulated by p53 out of a 19,000 probe array. Our data reveal that p53
not only induces genes known to be involved in growth arrest and apoptosis, but also activates
multiple genes that function to promote cellular growth, supporting the role of p53 as a dual-
signal gatekeeper in balancing apoptosis with cellular growth. Strikingly, we show that the
majority of p53-responsive genes are repressed rather than activated and that gene repression by
p53 on a genomic scale is tightly linked to its ability to induce apoptosis. Extending these
observations, we show that p53 can repress the transcription of PLK, CHEK1 and PTTG1 directly or indirectly and their expression is required for maintaining cell survival. Thus, p53 utilizes both transcriptional activation and repression of its target genes for the full induction of apoptosis.

**Experimental procedures**

*Cell culture and drug treatments*

Human colon cancer cell line HCT116 and its derived isogenic p53 (-/-) cell line were kindly provided by Dr. Bert Vogelstein, Johns Hopkins University, Baltimore, MD. Cells were cultured in DMEM containing 10% FCS. All of the culture reagents and media were from Invitrogen. 5-Fluorouracil and Cycloheximide were purchased from Sigma.

*Western blotting*

Cell lysates were prepared as reported previously (25). Briefly, cells were lysed with cell lysis buffer [0.3% NP40, 1 mM EDTA, 50 mM Tris-HCl (pH 7.4), 2 mM EGTA, 1% Triton X-100, 150 mM NaCl, 25 mM NaF, 1 mM Na3VO4, 2 mM AEBSF, 5 µg/ml aprotinin, 1 µg/ml leupeptin] for 30 min on ice, and the lysates were clarified by centrifugation at 12,000 xg for 15 min at 4°C. Protein concentration was quantified (Bio-Rad, Hercules, CA) and protein samples (50 µg) were separated by SDS/PAGE and transferred onto immobilon membranes (Millipore, Bedford, MA). p53, PLK, PTTG1 and Chk1 proteins were identified using anti-p53, anti-Chk1 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-PLK and anti-PTTG1 primary antibodies (Zymed Laboratories).

*Apoptosis analyses*
Apoptosis was measured using FACS analysis of cells in sub-G1 phase. Cells were harvested and fixed in 70% ethanol. The fixed cells were then stained with propidium iodide (50µg/ml) after treatment with RNase (100 µg/ml). The stained cells were analyzed for DNA content by fluorescence-activated cell sorting (FACS) in a FACScaliber (Becton Dickinson Instrument, San Jose, CA). Cell cycle fractions were quantified with CellQuest (Becton Dickinson).

Microarray Hybridization and Data Analysis

Total RNA was extracted with the use of Trizol reagent (Invitrogen, Carlsbad, CA) and the Qiagen RNAease Mini kit according to the manufacture’s instructions (Valencia, CA). The methods for probe labeling reaction and microarray hybridization were described previously (26) except the total RNA was used directly for labeling. For all experiments, universal human reference RNA (UHR) (Stratagene, La Jolla, CA) was used to generate a reference probe for drug treated and untreated samples. 30 µg of total RNA from experimental samples or equal amount of UHR were labeled with Cy5 and Cy3, respectively, by using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). The microarray hybridization, image process, and data normalization were as described previously (26). The log 2 ratios of each time point were then normalized for each gene to that of untreated cells (time 0) to obtain the relative expression pattern. The genes that showed substantial differences after drug treatment were selected based on a 2-fold change of expression value for at least two time points across all experiment conditions. A total of 1260 of ~19,000 genes met the criteria and were further analyzed using clustering and display programs (rana.stanford.edu/software) developed by Eisen et al (27).

Real-Time Quantitative RT-PCR
Total RNA was extracted using RNeasy kit (Qiagen). 100 ng of total RNA from each sample was subjected to real time RT-PCR using ABI PRISM 7900 Sequence Detection System and SYBR Green master mix (Qiagen) according to manufacturer’s protocol. Primers are available upon request. β-actin was used as an internal control for equal amount of RNA used.

Luciferase Reporter Gene Assay

For promoter reporter constructs, DNA fragments containing approximately 1-1.5 kb 5′-flanking region of respective genes were isolated by genomic PCR and subsequently subcloned into pGL3-basic vector (Promega, Madison, WI). The PCR primers are as follows: for PLK1, CCGGGGTACCTGCTGTAAATGTTTACAATGG (forward) and GGAAGATCTCTGGGAACGTTACAAAAGCCT (reverse); for CHEK1, TCCCCCGGG ACCGGCTGAAGTAAAGCAT (forward) and CCCAAGCTTCTCCCAAGCACACCGAAGGT (reverse); for PTTG1, CCGGGTACC GCAAAATTTCTTTTCATATCTG (forward) and CCCAAGCTT TGGGGTCTTTAGAGGTCTCC (reverse). The wild-type p53 (pCMV-p53) and dominant negative mutant p53 (pCMV-p53R175H) expression vectors were obtained from Dr. B. Vogelstein. For transfection, 1 x 10^4 HCT116 p53 (-/-) cells were seeded in triplicates into 96-well tissue culture plates and transfected with Fugene 6™ (Roche) according to manufacture’s instructions. For each well, 60 ng of reporter construct or PGL3 basic empty vector, were co-transfected with 1 or 10 ng of wt or mt p53 expression vectors, together with 3 ng of Renilla luciferase control vector PRL-null (Promega). The total amount of transfected DNA in each well was kept constant by adding empty vector pCMV plasmid. To correct for variation in transfection efficiency, reporter firefly luciferase activity was normalized to Renilla luciferase activity, which was measured using DLR (Dual-Luciferase Reporter Assay System) kit (Promega) according to the manufacturer's instructions.
**Chromatin immunoprecipitation (ChIP) assay**

ChIP assays with HCT116 cells were carried out as described in Weinmann et al (28) and Wells and Farnham (29). Briefly, cells at different time point before and after 5-FU treatment were crosslinked with 1 % formaldehyde for 10 mins at room temperature. Formaldehyde was inactivated by addition of 125 mM Glycine. Chromatin extracts containing DNA fragment of average size of 500 bp were immunoprecipitated using anti-p53 DO1 monoclonal antibody (Santa Cruz). For all ChIP experiments, quantitative PCR analyses were performed in real time using ABI PRISM 7900 Sequence Detection System and SYBR Green master mix as described (30). Relative occupancy values were calculated by determining the apparent immunoprecipitation efficiency (ratios of the amount of immunoprecipitated DNA over that of the input sample) and normalized to the level observed at a control region, which was defined as 1.0. The control region is a 279 bp region on chromosome 22 and is amplified using the following primers: 5’-GGACTCGGAAGAGGTTACCTCGGG-3’ and 5’-GTCGCCTCCGGCTTGACTGACTAATTC-3’. The error for independent determinations is ±10%.

List of primers:

**CHEK1**: A, GAAATTTGCAGGCTTTCCTTCTCGTATTC (forward) and CCTACCTCAGCATCCCAAGTCACTG (reverse); B, CGGAGAGGTTTCTAATTAATCTTTCTCGTCGCTT (forward) and CGGGGATGACAAAATATAGTTTAATG (reverse); C, AAGCTCAACATTAACCTCGTCTTC (forward) and GTGCTTTTGAACCTCGAGGTGTTACT (reverse).

**PTTG1**: A, TCCCGTCGCCTCGCAAGTGAATAAT (forward) and CCCGCCAGGAAATAGTGCGCATG (reverse); B, CCTTCCTCTCATCCTACTAATACCT (forward) and CGAAAGGCTTTGACACTACCTC (reverse); C,
CCCAGAAAACGTGCCACAAAGTTTGCAAG (forward) and
TCACGCAGGTCTTAACAGCCGCATTCA (reverse).

siRNA and Transfection

siRNAs were synthesized by in vitro transcription method using Silencer™ siRNA Construction Kit (Ambion) following the manufacture’s instruction. Targeting sequence for human PLK (accession no. NM_005030): AAGGGCGGCTTTGCCAAGTGC; and for PTTG1 (accession no. NM_004219): AAAGCTCTGTTCCTGCCTCAG. An unrelated siRNA targeting a sequence in GADPH mRNA (Ambion) served as a control. Cells were seeded in 6-well plates on the day before transfection at the concentration of $1 \times 10^5$ cells per well. Cells were transfected with siRNAs at a concentration of 30 nM using Oligofectamine reagent (Invitrogen) in serum-free DMEM. After incubation at 37 °C for 4 h, DMEM containing 20 % FCS was added. Cells were harvested 48 h after transfection for protein expression or FACS analysis.

Results:

Genome-wide effects in gene expression in response to 5-FU treatment in HCT116 and HCT116 p53 (-/-) cells

To study the regulation of gene expression by p53 during genotoxic drug-induced apoptosis, we treated the well-characterized colorectal cancer HCT116 cells and the p53-deficient subline with 5-FU, an antimetabolite anticancer drug known to induce p53-dependent apoptosis in these cells (24). Addition of 5-FU to the culture medium of HCT116 cells induced a strong p53 accumulation and apoptosis in a time-dependent manner. However, only a minimum apoptosis was induced in their p53 null counterparts (Fig.1, A & B). Thus, as previously reported, 5-FU induces a p53-dependent apoptosis in HCT116 cells. We then used spotted Oligo arrays...
representing 19 k gene set (Genome Institute of Singapore) to study the temporal gene expression of p53 wild-type and null HCT116 cells following 5-FU treatment at different time points. The expression of approximately 1260 genes changed significantly (2.0-fold cut off) after 5-FU treatment for at least 2 time points after we applied a series of filters and normalizations described in Materials and Methods. A hierarchical clustering method was used to group drug responsive genes on the basis of similarities in their expression patterns (27) and three clusters consisting of 280 genes display progressive changes in gene expression across the time points, as illustrated in Fig. 1C. When gene expression patterns in HCT116 wild type cells were compared with HCT116 p53 (-/-) cells at 2, 4, 6, 8, 12 or 24 h after treatment, two clusters of genes were found to be differentially expressed between HCT116 and HCT116 p53 (-/-) cells both as a function of time following drug treatment and p53 status (Fig. 1C, cluster B & C). The vast majority of genes were down-regulated by 5-FU treatment in HCT116 cells: cluster B & C show 41 genes that were up-regulated whereas 239 genes that were down-regulated with induction occurring temporally earlier than repression (Fig. 1D). However, in HCT116 p53 (-/-) cells treated with 5-FU, these gene responses were either abolished or largely reduced suggesting that the major transcriptional effects by 5-FU are mediated by p53. A much smaller subset of genes were concomitantly increased following 5-FU treatment in both p53 wild-type and null HCT116 cells (Fig.1C, Cluster A). One gene of note in this cluster is Cyclin E2, which was significantly induced by 5-FU (~3 fold), and Cyclin E1, to a lesser degree (~ 1.5 fold). These genes may represent the general response to 5-FU and appeared to be independent of p53 status. The array results were confirmed in a select subset of these genes by real time RT-PCR (Fig. 2).

**Genes affected by levels of p53 protein accumulation**

Protein synthesis inhibitor Cycloheximide (CHX) has been shown to block p53 accumulation and to inhibit p53-dependent transcription (31). To identify genes whose
expression is directly influenced by p53 protein levels, we pretreated the culture with CHX for 30 min prior to the induction by addition of 5-FU and harvested the cells at different time points. As predicted, pretreatment of HCT116 cells with 10 µg/ml CHX significantly abrogated 5-FU-induced p53 accumulation (Fig. 3A) and accordingly abolished 5-FU-induced apoptosis by 90 % (Fig. 3B). However, CHX treatment had negligible effect on low level of apoptosis observed in 5-FU-treated HCT116 p53 (-/-) cells (data not shown). This observation suggests the abrogation of p53 accumulation by CHX blocks 5-FU-induced apoptosis in HCT116 cells. Of 41 genes that were induced following p53 accumulation in the absence of CHX, 38 were either abolished or largely reduced in their induction in the presence of CHX (Fig. 3C). At all the time points analyzed, the expression of majority of p53 inducible genes are directly associated with p53 protein levels. In contrast to the p53-inducible genes, we found that approximately 40% of p53-repressed genes (n=50) were insensitive to CHX treatment and therefore not p53 regulated (Fig. 3C). Thus, only those that are sensitive to blockade of p53 accumulation by CHX are considered truly p53 regulated genes. These genes are listed in Table 1. Note however, that since cycloheximide blocked p53 accumulation itself, we cannot discriminate between p53 primary and secondary target genes.

P53-inducible genes

Among 41 genes up-regulated by p53, 16 have been identified previously as p53 targets in a variety of systems (Table 1, bold text). They are putative p53 targets involved in apoptosis (PUMA, NOXA, PIG3, and FAS/CD95) or growth arrest (CDKN1A, GADD45, 14-3-3σ). Induction of other known p53 targets such as MDM2, APAF, and KILLER/DR5 were also observed but to a lesser degree than those listed in Table 1. The successful detection of large number of previously identified p53 targets indicates that our system is robust and accurate in identifying p53 responsive genes. However, 3 out of 41 p53-inducible genes including known
p53 targets *GADD45A* and *PPMID* (Fig. 3) were not sensitive to CHX treatment, indicating that these three genes must be regulated by p53 in a way that is distinct from other target genes. This observation seems to be in line with a previous report indicating that *GADD45A* is regulated by p53 through indirect transactivation (32). Of all p53-inducible genes, *CDKN1A* (encoding p21) was most strongly up-regulated (9-fold), which is consistent with a high binding affinity of p53 to p21 promoter (11). We also found that *PUMA* was most responsive among the detectable putative apoptotic targets induced by p53, supporting the previous reports that PUMA was a major apoptotic target mediating p53-induced apoptosis in colorectal cancer cells (33,34). We also identified 25 previously unidentified putative target genes up-regulated by p53. Of these 25, 19 (76%) were found to contain putative p53 binding sites in their promoter regions (data not shown). This compares to 29% in genes unresponsive to p53 induction. Interestingly, a candidate tumor suppressor gene *SERPINB5* was also strongly induced by p53 at 24 h after 5-FU treatment (9-fold), pointing to a potential role of its gene product in mediating p53-mediated tumor suppressor function. The other two candidate genes of interest are *DUSP5* &14, which encode dual specificity phosphatase 5 and 14, respectively. In line with these findings, a recent study shows that *DUSP2*, another member of this family, is a transcription target of p53 that functions in signaling apoptosis and growth arrest (15). Unexpectedly, we found that p53 activation also led to the induction of a number of genes associated with mitogenic responses. These genes include *TGFα, SEK, TOP1, CNK* and *EPHA2* and all contain putative p53 binding sites in their promoters. In particular, *TGFα* and *EPHA2* have been previously reported to be linked to the activation of MAPK signaling cascade that promotes cell growth (35,36). This implies that activation of p53 is accompanied by an induction of cellular mitogenic programme. This finding is consistent with recent studies showing that ability of p53 to induce apoptosis is counteracted by simultaneously augmenting opposing signals (37,38). As such, our identification of multiple
potential p53 target genes with growth-promoting functions is further evidence to support this notion.

**p53 repressed genes**

p53-dependent activation of apoptotic targets has been extensively studied. However, the role of p53-mediated transcriptional repression in the induction of apoptosis is less investigated and in particular, has never been explored in a genomic scale. We observed that p53-mediated transactivation was maximally induced as early as 6-8 h after 5-FU treatment but induction of apoptosis did not become obvious until 12 h later when gene repression by p53 reached maximal (Fig. 1D). To examine whether p53-mediated transcriptional repression contributes to the apoptosis induced by 5-FU, CHX was added to HCT116 cells 6 h after 5-FU treatment when p53-mediated transactivation had been fully induced. As anticipated, this treatment caused a significance decrease in p53 accumulation at 12 h and abrogated all the p53 accumulation at 24 and 48 h in comparison with 5-FU treatment alone (Fig. 4A). In this manner, we were able to separate the immediate effects of p53 as compared to the late effects. As a consequence, no change in gene induction was noted, whereas gene repression was collectively affected (Fig. 4 C & D). 5-FU plus CHX reduced apoptosis by 60% at 48 hours compared to 5-FU treatment alone. That no decrease in apoptosis was seen at 24 h implicates that p53-mediated transcriptional repression contributes to apoptosis later in the time course (Fig. 4B). Hence, the overall ability of p53 in inducing apoptosis appears to be tightly associated with its ability to repress gene expression. Among p53 repressed genes are those that function in mitosis (*PLK, PTTG1, CHEK1, CDC20, CDC25B, CCNB1&2*), and DNA replication and repair (*MCMs, H2AX, NBS1, and RFC4*) (Table 1). Previously known p53 repressed genes such as *HSP70, CCNB2, TOP2A* (39,40) were also included.
p53 represses the expression of PLK, PTTG1, and CHEK1 either directly or indirectly.

To gain further mechanistic insights of p53-dependent down-regulation of its target genes and to determine whether their down-regulation contributes to apoptosis, we selected three genes, PTTG1, PLK and CHEK1 for further evaluation because of their known importance in colorectal cancer biology and cell cycle checkpoint regulation (41-44). To further confirm the microarray data, we performed western blots to examine the effect on protein levels by p53. As anticipated, PLK, PTTG1 and Chk1 proteins were preferentially repressed in response to 5-FU in a time-dependent manner in HCT116 cells in comparison with HCT116 p53 (-/-) cells (Fig. 5A).

To determine whether their promoter sequences were responsive to p53, genomic fragments of ~1 kb from 5'-flanking region of PLK, PTTG1 or CHEK1 were isolated and subcloned into a luciferase reporter plasmid. The constructs were then cotransfected into p53-deficient HCT116 cells with either an empty vector (pCMV), or increasing concentrations of wild-type p53 (pCMV-p53) or mutant p53 (pCMV-p53-R175H) expression plasmids and a normalization control. We found that wild-type p53 transfection strongly repressed all the three reporter activities in a dose-dependent manner. In contrast, the reporters were unresponsive to the p53-R175H mutant (Fig. 5B). These promoters were quite sensitive to p53-dependent repression since as little as 1 ng of wild-type p53 construct was sufficient to repress more than 50% of promoter activity derived from 60 ng reporter construct. These data indicate that p53 might down regulate the expression of PLK, PTTG1 and CHEK1 by targeting their promoters.

We next examined whether p53 binds directly to the PTTG1, PLK1 and CHEK1 promoters since sequence analysis revealed several putative p53 binding sites in their promoters. We used p53 antibody to immunoprecipitate chromatin from HCT116 cells treated with 5-FU for different periods of time. The ChIP assays showed that one out of 3 tested genomic fragments containing p53 binding site from CHEK1 promoter was enriched after immunoprecipitation during the course of 5-FU treatment, reaching 6- to 7- fold at the 24 hours time-point (Figure 6,
upper panel). This fragment is located from nt-1429 to -1169. On the other hand, two out of 3 tested genomic fragments from PTTG1 promoter were found to be enriched following 5-FU treatment. The identified regions that show positive p53 binding are from nt -2624 to -2872 and from +102 to -177, respectively (Fig 6, lower panel). Importantly, the kinetics of p53 bindings to both PTTG1 and CHEK1 promoters are consistent with that of PTTG1 and CHEK1 RNA transcript levels following 5-FU treatment. Hence, the level of p53 binding inversely correlates with the accumulation of RNA transcripts. Together with the promoter assay, these results demonstrate that p53 interacts directly with p53-binding sites in CHEK1 and PTTG1 gene promoters and negatively regulate their transcription. However, we did not detect any p53 binding to the PLK1 promoter (data not shown). Therefore, PLK1 promoter might be regulated by p53 through a mechanism that does not require direct p53 binding.

RNAi-mediated knockdown of PLK or PTTG1 directly causes apoptosis

p53 repressed gene targets BCL-2 and survivin have been shown to be anti-apoptotic (4,17,45). To test whether PLK and PTTG1 are required for maintaining cell survival or anti-apoptotic, we used small interference RNA (siRNA) to specifically reduce their expression in HCT116 cells. Following transfection with siRNA for PLK, the level of PLK protein fell by more than 80% after 48 h (Fig. 7A). PTTG1 protein expression was similarly effectively reduced by PTTG1 siRNA. Consequently, HCT116 cells transfected with PLK or PTTG1 siRNAs showed a significant increase (~100%) in apoptosis in comparison to cells transfected with unrelated control siRNA (Fig. 7B). In addition, cells depleted of PLK or PTTG1, were more sensitive to 5-FU treatment compared to cells treated with unrelated control siRNA and this effect appeared to be more pronounced in HCT116 p53 (-/-) cells (data not shown) suggesting that inhibition of PLK or PTTG1 was sufficient to induce apoptosis in the absence of p53.
Together, these observations indicate that individual p53-repressed genes, such as PLK and PTTG1, appear to be important in maintaining the cell survival.

**Discussion**

Although a number of p53 targets have been identified and multiple cellular events involving p53 continue to increase in complexity, the entire picture of p53-regulated transcription in the regulation of apoptosis has not been fully explored. HCT116 cells exhibiting p53-dependent apoptosis in response to anticancer drug 5-FU provides a model system for identifying molecular events important in p53-mediated apoptosis of tumor cells. Using this system and a genome-wide approach, we have identified approximately 230 out of 19,000 genes responsive to 5-FU in a p53-dependent manner using a stringent selection algorithm. Validating this approach for discovery of novel targets regulated by p53, we have identified more than 20 previously known p53 target genes in our study.

There is now compelling evidence that the transcriptional activity of tumour suppressor p53 is required for its growth suppressing and tumour suppressing activities. Most studies have focused on the transactivation function of p53 because of the strong association between transactivation and tumour suppression. However, p53 is also able to repress gene expression but the functional consequence of this repression is largely unexplored. In this study, we show that p53 regulates the majority of gene transcription through transcriptional repression. We further demonstrate that transcriptional repression by p53 on a genomic scale is important for its ability to promote apoptosis since selective abrogation of gene repression function of p53 significantly diminishes the apoptotic response. As such, p53 may regulate apoptosis through a coordinated programme that includes both the activation of apoptotic genes (e.g., *PUMA*, *NOXA*, *PIG3*, *FAS/CD95* and *DUSP2*) and the repression of potential survival genes (e.g., *PLK*, *PTTG1*, or *CHEK1*). Consistent with our observation is a recent report in which 80% of the p53-responsive
genes have been found to be repressed rather than activated (17). Intriguingly, we found many genes involved in the regulation of chromatin segregation (PTTG1 &3, H2AX), mitosis (PLK, KNSL), cell cycle checkpoint (CHEK1, NBS1, CDC25B), DNA replication (MCMs, TOP2A. RFC) were repressed by p53; suggesting p53 is indeed a master transcription factor coordinately controlling cell growth and cell death.

In contrast to transactivating genes via binding to the consensus sequence in the promoter region of target genes, the mechanisms underlining the p53-mediated transrepression are largely unknown. Genes repressed by p53 represent either direct targets of p53 or those repressed as a consequence of changes in other transcriptional modulators. Although our gene expression data does not necessarily identify the direct targets of p53, we used chromatin immunoprecipitation and reporter assays to demonstrate that PTTG1 and CHEK1 are direct targets of p53, whereas PLK1 might be regulated by p53 through an indirect mechanism. Of note, while our project is in progress, a study showing that p53 transcriptionally represses PTTG1 was reported (46). Our observation is in agreement with the current notion that p53 represses target gene expression through both direct or indirect mechanism. For instance, p53 had been found to repress survivin expression through binding to a p53 consensus sequence overlapping with an E2F1 binding site (4). Alternatively, p53 can down regulate its target gene expression indirectly through recruiting a repressor complex via p21. Genes associated with similar functions with similar expression kinetics might share common regulatory elements or modules. Interestingly, multiple p53 repressed genes identified in this study including PLK, PTTG1, CHEK1, and MCMs are potential targets of E2F family as reported in a recent study (47). Thus, these observations raise an intriguing possibility that p53 might repress target gene expression through interfering E2F-mediated transcription as a principle mechanism.

Regardless of the mechanism, genes down-regulated by p53 primarily appear to include those involved in maintaining cell growth. We show that PLK, PTTG1 and CHEK1 are potential
targets of p53 and inhibition of PLK and PTTG1 transcription by RNAi led to a decrease in cell viability (48,49). Indeed, several p53 repressed genes including PLK 1 and PTTG1 have been found to be over-expressed in primary colorectal cancers (41,42,46,50) or related to the histological grades of cancer (Lance Miller, personal communication). Thus, identification of numerous putative targets repressed by p53 will be beneficial in novel drug development that might be used alone or combined with conventional chemotherapy to maximize the efficacy of anticancer drug treatment.

Among genes induced by p53 activation, we found unexpectedly that p53 is able to induce multiple genes participating in cell proliferation or pro-survival pathways. Genes included in this category include early-growth-response genes SNK and CNK, also known as immediate-early genes that play important roles in regulating cell proliferation (51-53). We also found that TGFα, a major ligand for EGF receptor in cancer tissues (54), and EPHA2, a tyrosine receptor kinase, are potential p53 targets and both are linked to the regulation of Ras/MEK/MAP kinase pathway that promotes proliferation (35,36). Identification of growth-related genes as potential p53 targets is consistent with the recent reports that p53 also functions under some conditions to promote cellular growth through its target gene such as HB-EGF (55). Thus, given the opposing signals induced by p53 activation, it seems that the concept of antagonistic pleiotropy involving dual signals of growth and death might also apply to p53, as it does to oncogenes such as MYC and RAS (56). Potentially attenuation of p53 activation of growth promoting genes might permit the molecular modulation of p53 apoptotic function, which will be useful in augmenting chemotherapeutic responsiveness.
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Figure Legends

Figure 1. Microarray analysis of gene expression profiles in colorectal cancer HCT116 and HCT116 p53 (-/-) cells in response to 5-FU treatment. (A) 5-FU induces p53-dependent apoptosis in HCT116 cells. HCT116 and HCT116 p53 (-/-) cells were treated with 5-FU (375 µM) and harvested at different time points as indicated. Apoptosis was evaluated by sub-G1 analysis using FACS. (B) p53 accumulation following 5-FU treatment. (C) Clustering analysis depicting genes that are regulated dependently and independently of p53. Cluster and Tree Viewer (27) were used to cluster 280 genes showing progressive changes (2-fold for two time points) in gene expression into three clusters. Red and green represent up-regulation and down-regulation, respectively, relative to the untreated control (black). The intensity of color correlates to the magnitude of change. Selected genes in each cluster are shown. (D) Graphs showing the kinetics of gene expression response in a p53-dependent manner in Cluster B (upper panel) and Cluster C (lower panel). Each time point represents an averaged expression ratio (log 2) of these genes.

Figure 2. Real time RT-PCR analyses of selected genes. HCT116 and HCT116 p53 (-/-) cells were either untreated or treated with 5-FU and mRNA expression of selected genes measured by real-time RT-PCR using ABI 7900 Sequence Detection System and SYBR Green master mix. Representative genes whose expression are independently of p53 (CCNE2, upper panel); up-regulated by p53 (CNK1A, TGFα, and EPHA2, middle panel); down-regulated by p53 (PTTG1, RAD21, and CDKN3, lower panel) are shown. Fold changes relative to the untreated control (C, arbitrarily set as 1) at indicated times in HCT116 cells (open bar) and HCT116 p53 (-/-) cells (dark bar) are shown.
Figure 3. Cycloheximide (CHX) blocks p53 accumulation, apoptosis and p53-dependent transcriptional response. HCT116 cells were pretreated with or without CHX for 30 minus (CHX-0.5 h) before addition of 5-FU. Cells were harvested as indicated time points as in Fig. 1. (A) Western blots analyses of p53 protein levels in the presence or absence of CHX. (B) Apoptosis induced by 5-FU in the presence or absence of CHX. (C) Clustering analysis of gene responses in the presence or absence of CHX. (D) The expression kinetics representing the averaged fold of induction (log 2) for each time point in the presence or absence of CHX. Upper panel, p53 inducible genes; lower panel, p53 repressed genes that are sensitive to CHX.

Figure 4. Selective inhibition of p53-mediated transcriptional repression reduces 5-FU-induced apoptosis. HCT116 cells were treated 5-FU for 6 h before addition of Cycloheximide (CHX+6h). Cells were harvested at indicated time points as in Fig. 1 for p53 protein levels (A); apoptosis (B); and microarray analyses (C and D).

Figure 5. p53 induction represses PLK, PTTG1, and CHEK1 transcription. (A) 5-FU treatment reduces PLK, CHK1 and PTTG1 protein expression preferentially in HCT116 cells but not in HCT116 p53 (-/-) cells. Western blots analyses of total cell extracts treated with 5-FU at indicated times with specific antibodies as indicated. (B) PLK, PTTG1 and CHEK1 promoter-driven transcription is repressed by wild-type p53, but not by mutant p53. HCT116 p53 (-/-) cells were co-transfected with PLK, CHEK1 or PTTG1 luciferase reporter constructs with either pCMV-wtp53 (blank bar) or pCMV-mtp53(R175H) (dark bar) expressing vector. A reporter construct containing the CDKN1 promoter was also included as a positive control for the assay. After 24 h, cells were lysed and luciferase activity was measured as a relative value of measured luminescence from firefly luciferase and Renilla luciferase from cotransfected pRL-null (internal
control). Reporter activity induced by empty vector control was arbitrarily set as 1. Values represent the mean and variation of a typical experiment performed in triplicates.

**Figure 6. p53 binds directly to CHEK1 and PTTG1 promoters.** HCT116 cells were treated with 5-FU and harvested at indicated time points. ChIP was performed using p53 antibody. Levels of p53 binding (fold enrichment over a control region on chromosome 22) at CHEK1 (upper panel) and PTTG1 (lower panel) promoters at different time-points were determined by chromatin immunoprecipitation assays. Regions (A, B, C) surveyed containing putative p53 binding sites (rectangles) are indicated. Positions of the fragments that are positive for p53 binding are also shown.

**Figure 7. Depletion of expression of PLK or PTTG1 induces apoptosis.** (A) HCT116 cells were transfected with either unrelated control siRNA, PLK or PTTG1 siRNA (30 nM) for 48 h. A fraction of cells were lysed and tested by western blotting for PLK and PTTG1 expression. NS, nonspecific band. (B) PLK and PTTG1 siRNA treatments led to increased apoptosis in HCT116 cells, as measured by FACS analyses. Representative FACS analyses are displayed. (C) Graph showing relative cell death as compared to the control (Student’s t-test, \( P<0.001 \)). Background cell death induced by unrelated control siRNA was arbitrarily set to 1. Bars indicate mean and s.d. from at least three independent experiments.
Table 1. Genes regulated by 5-FU in p53-dependent manner

| Acc. Number | Gene Name | Description | 6 h | 8 h | 12 h | 24 h |
|-------------|-----------|-------------|-----|-----|------|------|
| NM_004086   | PLAB      | prostate differentiation factor | 4.02 | 7.10 | 6.56 | 5.07 |
| NM_014415   | PA26      | p53 regulated PA26 nuclear protein | 4.22 | 5.45 | 1.39 | 2.12 |
| AL13074     | TP53IP1   | tumor protein p53 inducible nuclear protein 1 | 4.02 | 5.29 | 2.61 | 2.00 |
| NM_019909   | LOC52024  | hypothesized protein | 4.48 | 3.98 | 2.35 | 1.59 |
| US2987      | PUMA      | Bcl-2 binding component 3 | 3.66 | 3.90 | 2.03 | 1.88 |
| NM_004024   | AUF3      | activating transcription factor 3 | 2.56 | 3.87 | 1.49 | 2.42 |
| D90070      | PMAIP1    | phospholipid-acyltransferase 3-acetyl-induced protein 1, NOXA | 3.34 | 3.86 | 2.04 | 2.03 |
| NM_003236   | TGF4      | transforming growth factor, alpha | 2.41 | 3.82 | 1.75 | 1.79 |
| NM_006763   | BTG2      | BTG family, member 2 | 3.17 | 3.76 | 1.68 | 2.04 |
| NM_006142   | SFN       | IκB-κ-3 | 2.02 | 3.57 | 3.81 | 2.86 |
| NM_005497   | GJA1      | gap junction protein, alpha 7, 45KD (connexin 45) | 1.95 | 3.15 | 2.35 | 1.41 |
| NM_020735   | C12orf5   | chromosome 12 open reading frame 5 | 2.38 | 3.03 | 1.00 | 1.53 |
| NM_003632   | PPM1D     | protein phosphatase 1D magnesium-dependent, delta isoform | 2.23 | 2.86 | 1.40 | 1.52 |
| NM_001376   | SE11      | Cdk4-binding protein p34SE11 | 3.17 | 2.85 | 2.35 | 1.73 |
| NM_008023   | BRKR2     | braylakin receptor B2 | 1.57 | 2.83 | 2.60 | 2.13 |
| NM_008043   | TNNFRSF4  | tumor necrosis factor receptor superfamily, member 6, FAS | 1.66 | 2.79 | 2.13 | 2.76 |
| NM_004073   | CNK       | cytokine-inducible kinase | 3.19 | 2.75 | 2.79 | 1.49 |
| NM_004881   | PGH3      | quinone oxidoreductase homolog | 1.68 | 2.68 | 1.78 | 1.85 |
| NM_004110   | FDXR      | ferredoxin reductase | 1.15 | 2.68 | 2.24 | 1.22 |
| NM_004431   | EPHA2     | Epha2 | 1.45 | 2.65 | 1.64 | 1.80 |
| NM_004419   | DUO5P5    | dual specificity phosphatase 5 | 1.22 | 2.57 | 0.74 | 2.14 |
| NM_001924   | GADD45A   | growth arrest and DNA-damage-inducible, alpha | 3.03 | 2.50 | 2.12 | 1.91 |
| AB036063    | RRM2B     | ribonucleotide reductase M2 B (TP53 inducible) | 1.20 | 2.50 | 0.87 | 2.57 |
| NM_016545   | HR5       | immediate early response 5 | 1.87 | 2.44 | 1.47 | 2.35 |
| NM_005749   | T0B1      | transducer of ERBB2, 1 | 2.39 | 2.43 | 2.63 | 2.08 |
| NM_007026   | DUO5P4    | dual specificity phosphatase 14 | 1.74 | 2.42 | 2.66 | 3.02 |
| NM0643      | ID2B      | striated muscle contraction regulatory protein | 2.36 | 2.19 | 1.35 | 0.95 |
| NM_003840   | TNNFRSF1D | tumor necrosis factor receptor superfamily, member 10d, | 2.37 | 2.19 | 0.99 | 1.79 |
| NM_004148   | NN1I      | ninjurin I | 2.31 | 2.10 | 2.06 | 2.15 |
| NM_005202   | RPS27L    | ribosomal protein S27-like | 1.93 | 2.03 | 1.69 | 2.57 |
| NM_006622   | SNE       | ornithine decarboxylase | 1.40 | 2.01 | 1.12 | 2.73 |
| AK025640    | SES2      | steatin 2 | 1.53 | 1.90 | 1.47 | 1.26 |
| AL136549    | PIR121    | cytoplasmic FMRF interacting protein 2 | 1.20 | 1.70 | 2.14 | 2.09 |
| NM_006404   | PROC      | protein C receptor, endothelial (EPCR) | 1.33 | 1.43 | 2.06 | 2.29 |
| NM_005978   | ST26A2    | S100 calcium binding protein A2 | 1.10 | 1.30 | 2.08 | 2.68 |
| NM_002839   | SERPINB5  | serine (or cysteine) protease inhibitor, clade B (ovalbumin), member 5 | 1.00 | 1.00 | 4.34 | 9.95 |
| NM_002023   | FMOD      | fibromodulin | 1.00 | 1.00 | 2.58 | 3.00 |

Genes downregulated

| NM_006294   | UQCRB     | ubiquinol-cytochrome c reductase binding protein | -2.44 | -2.17 |
| NM_002497   | NEE2      | NIMA (never in mitosis gene a)-related kinase 2 | -2.50 | -2.50 | -1.39 | -1.75 |
| NM_003360   | UGT8      | UDP glycosyltransferase 8 (UDP-galactose ceramide galactosyltransferase) | -2.94 | -3.13 | -1.04 | -1.04 |
| NM_005787   | NQ5T6L    | Nq5t6 (D. melanogaster)-like protein | -1.14 | -3.13 | -2.08 | -1.69 |
| NM_001137   | FAM      | fumarlylacetate hydratase (fumarylacetocoxylase) | -0.84 | -3.03 | -3.03 | -1.01 |
| NM_002497   | ADAMS9    | a disintegrin-like and metalloprotease (repellin type) with thrombospondin type 1 motif, 9 | -2.00 | -1.64 | -3.03 | -2.08 |
| NM_004916   | KIAAD05   | Wits’ tumour 1-associating protein | -2.08 | -1.59 | -2.63 | -2.04 |
| NM_001067   | TOP2A     | topoisomerase (DNA) II alpha (170KD) | -2.22 | -1.35 | -2.94 | -1.47 |
| NM_004616   | DEEPEST   | mitotic spindle coiled-coil related protein | -2.56 | -1.14 | -2.44 | -1.49 |
| NM_003554   | SERPINa7  | serine (or cysteine) protease inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7 | -2.33 | -1.12 | -2.78 | -1.49 |
| NM_003390   | WEE1      | WEE1+ homolog (S. pombe) | -2.04 | -1.35 | -2.86 | -1.69 |
| NM_004303   | PAF1A1B1   | platelet-activating factor acylhydrolase, isoforn Bb, subunit (45KD) | -2.00 | -1.37 | -4.17 | -1.56 |
| NM_002110   | PPP1CC     | protein phosphatase 1, catalytic subunit, gamma isoform | -2.22 | -1.25 | -3.57 | -1.45 |
| NM_006330   | LYP1A1     | lysophospholipase A1 | -2.04 | -1.52 | -2.78 | -1.10 |
| D16815      | NR1D2     | nuclear receptor subfamily 1, group D, member 2 | -2.27 | -1.45 | -3.03 | -1.08 |
| Acc. Number | Gene Name | Description                                      | Fold Change |
|------------|-----------|--------------------------------------------------|-------------|
| NM_004589  | CLTC      | clathrin, heavy polypeptide (Hc)                 | -2.94       |
| NM_001304  | CPD       | carboxyesterase D                               | -2.08       |
| AB007896   | KIAA0436  | putative L-type neutral amino acid transporter   | -1.79       |
| AK0031872  | PD2L      | programmed death ligand 2                       | -1.12       |
| NM_002740  | PRKCI     | protein kinase C, iota                          | -1.89       |
| NM_005566  | LDHA      | lactate dehydrogenase A                         | -1.96       |
| NM_005030  | PLK       | polo-like kinase (Drosophila)                   | -1.69       |
| NM_004219  | PTTG1     | putative tumor-transforming 1                    | -2.08       |
| NM_014473  | HSA0761   | putative dimethyladenosine transferase           | -1.82       |
| NM_002424  | KNSL7     | kinesin-like 7                                  | -2.08       |
| NM_003431  | CAD       | carbamoyl-phosphate synthetase 2                | -2.33       |
| NM_016885  | ANLN      | anillin, actin binding protein (scaps homolog, Drosophila) | -1.85       |
| NM_002866  | KPN1A2    | karyophycin alpha 2 (RAF1, importin alpha 1)    | -3.85       |
| NM_004761  | CCNB2     | cyclin B2                                       | -2.08       |
| NM_001492  | GDF1      | growth differentiation factor 1                 | -2.13       |
| NM_003234  | TFRC      | transferin receptor (p0, CDF1)                  | -2.08       |
| NM_004865  | HEPF1D1   | homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain 1 | -2.22       |
| M25753     | CCNB1     | cyclin B1                                       | -1.72       |
| NM_001255  | CDC20     | CDC20 cell division cycle 20 homolog (S. cerevisiae) | -1.59       |
| R0404     | HPX       | hemopexin                                       | -1.32       |
| AP005289   | PTTG3     | putative tumor-transforming 3                    | -1.85       |
| NM_006575  | MAP461    | minigen-activated protein kinase kinase kinase 5 | -2.44       |
| NM_012484  | HMRR      | hyaluronan-mediated motility receptor (HMRR)     | -2.27       |
| NM_005192  | CDK3      | cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase) | -1.79       |
| NM_005465  | TRIOAP    | trophinin associated protein (tassin)           | -2.08       |
| NM_005960  | LOC51076  | CGL-32 protein                                  | -3.45       |
| U08743     | EPHA5     | EPHA binding protein p400                       | -1.37       |
| NM_007934  | PHIP      | pleckstrin homology domain interacting protein   | -3.33       |
| NM_002153  | EHF       | ets homologous factor                           | -3.33       |
| X67155     | KNSL5     | kinesin-like 5 (motin kinesin-like protein 1)   | -2.78       |
| AP231458   | PRKCBP1   | protein kinase C binding protein 1               | -2.17       |
| AK026668   | Unknown   | unknown                                         | -2.56       |
| NM_001110  | ADAM30    | a disintegrin and metallopeptidase domain 10    | -1.41       |
| AK002802   | FLJ39140  | likely ortholog of mouse tubulin alpha 4        | -1.59       |
| NM_009284  | NBS1      | Nijmegen breakage syndrome (nibrin)             | -0.92       |
| L10284     | CAXX      | calaxin                                         | -1.67       |
| NM_002105  | H2AFX     | histone family, member X                        | -1.45       |
| NM_002916  | RFC4      | replication factor C (activator 1) 4 (37KD)     | -1.25       |
| NM_012145  | DFFMK     | deoxyribonuclease 1-like (thymidylate kinase)   | -1.43       |
| AP171055   | TR2       | thioredoxin reductase 2                         | -1.45       |
| NM_006904  | PRKDC     | protein kinase, DNA-activated, catalytic polypeptide | -1.14       |
| AP21787    | SIDS-106  | inorganic pyrophosphatase                       | -0.90       |
| NM_001274  | CHEK1     | checkpoint kinase 1                             | -0.85       |
| NM_002136  | HNRPA1    | heterogeneous nuclear ribonucleoprotein A1       | -0.87       |
| NM_001533  | HNRPL     | heterogeneous nuclear ribonucleoprotein L        | -0.95       |
| NM_003156  | GART      | guanosine triphosphate (GTP)-binding protein    | -0.75       |
| NM_002137  | HNRPA2B1  | heterogeneous nuclear ribonucleoprotein A2/B1    | -0.86       |
| D57516     | MCM7      | mismatched chromosome maintenance deficient 7 (S. cerevisiae) | -0.87     |
| NM_001629  | TRAP1     | heat shock protein 5                            | -0.95       |
| M80322     | MRE1A     | vesicle trafficking, beach and anchor containing | -1.06       |
| NM_002539  | ODC1      | ornithine decarboxylase 1                       | -0.96       |
| NM_005550  | HNRPH1    | heterogeneous nuclear ribonucleoprotein H1 (H)  | -1.18       |
| NM_004117  | FKBP5     | FK506 binding protein 5                         | -1.18       |
| NM_002882  | RANBP1    | RAN binding protein 1                           | -1.10       |
| JM_001678  | ATP5D     | ATP synthase                                    | -1.10       |
| NM_006436  | OXCT      | 3-oxoadipate CoA transferase                    | -1.15       |
| NM_004117  | FKBP5     | FK506 binding protein 5                         | -1.18       |
| NM_002882  | RANBP1    | RAN binding protein 1                           | -1.10       |
| NM_001678  | ATP5D     | ATP synthase                                    | -1.03       |
| NM_005915  | MCM6      | MCM6 mismatched chromosome maintenance deficient | -0.93       |
| AP035191   | NAP5       | nuclear autoantigenic sperm protein (histone-binding) | -0.93       |
| NM_002388  | MCM3      | MCM3 mismatched chromosome maintenance deficient 3 (S. cerevisiae) | -1.15       |
### Table 1. Continued

| Acc. Number | Gene Description | 6 h | 8 h | 12h | 24h |
|-------------|------------------|-----|-----|-----|-----|
| NM_002628   | profilin 2       | -1.04 | -1.39 |
| U20582      | actin-like protein | -1.19 | -1.33 | -2.00 | -2.13 |
| NM_004959   | spermine synthase | -1.23 | -1.37 | -3.45 | -2.17 |
| U30828      | splicing factor, arginine-ribose-rich 6 | -1.23 | -1.43 | -3.23 | -2.37 |
| NM_003310   | tumor suppressor subtransferable candidate 1 | -1.69 | -1.45 | -2.70 | -2.00 |
| NM_004483   | glycine cleavage system protein H (aminomethyl carrier) | -1.01 | -1.32 | -3.03 | -2.44 |
| NM_003133   | signal recognition particle 9KD | -1.39 | -1.61 | -2.94 | -2.33 |
| NM_004990   | methionine-tRNA synthetase | -1.45 | -1.82 | -2.44 | -2.50 |
| NM_014501   | ubiquitin carrier protein | -1.28 | -1.43 | -3.13 | -2.78 |
| NM_003136   | CSE1L | similar to rat nuclear ubiquitin conjugate kinase 2 | -1.19 | -1.45 | -2.38 | -2.22 |
| AK025313    | NUCCS | recombination protein REC14 | -1.28 | -1.49 | -2.22 | -2.08 |
| AF309553    | CYC1 | leucine-rich acidic protein-like | -1.12 | -1.61 | -2.70 | -2.33 |
| AK025624    | LANP-L | alanyl-tRNA synthetase | -1.16 | -1.79 | -3.03 | -2.44 |
| NM_014050   | AARS | polyadenylate binding protein-interacting protein 1 | -1.05 | -1.43 | -2.63 | -2.22 |
| NM_006441   | PAP1 | high-mobility group (nonhistone chromosomal) protein 1 | -1.14 | -1.54 | -2.17 | -2.04 |
| AL110194    | HMG1 | tyrosyl-tRNA synthetase | -1.52 | -1.61 | -2.44 | -2.00 |
| NM_003680   | YARS | splicing factor 3b, subunit 3, 130KD | -2.04 | -2.00 | -2.56 | -2.17 |
| NM_013286   | E46L | like mouse brain protein E46 | -1.67 | -1.75 | -4.00 | -3.03 |
| NM_002084   | GIP1 | G1 to S phase transition 1 | -1.49 | -1.89 | -3.33 | -2.13 |
| NM_004730   | ETF1 | enkaryotic translation termination factor 1 | -1.56 | -1.79 | -3.45 | -2.22 |
| NM_001483   | GBAS | ribosomal protein D15 | -1.47 | -1.52 | -3.70 | -2.38 |
| NM_001968   | EIF4E | signal-activated protein kinase catalytic subunit | -1.49 | -1.45 | -2.70 | -2.04 |
| NM_006388   | METAP2 | methionyl-tRNA synthetase | -1.59 | -1.47 | -3.03 | -2.00 |
| NM_004184   | WARS | protein kinase, AMP-activated, gamma 2 non-catalytic subunit | -1.59 | -1.75 | -3.33 | -2.17 |
| D38438      | PMSL2 | protein kinase, AMP-activated, gamma 2 non-catalytic subunit | -1.59 | -1.54 | -2.64 | -2.17 |
| NM_006666   | ACY1 | Win-AaCyt 3B1 | 1.54 | -1.47 | -2.44 | -2.33 |
| NM_007009   | RPL10A | ribosomal protein L10a | -1.02 | -0.84 | -3.33 | -3.13 |
| NM_005953   | TBCD | ribosomal protein D15 | -1.67 | -1.11 | -2.22 | -3.13 |
| NM_005412   | SHMT2 | RecQ protein-like 4 | -1.54 | -1.54 | -2.63 | -2.88 |
| NM_004820   | RECQ4 | G1 to S phase transition 1 | -1.45 | -1.75 | -2.00 | -2.38 |
| DS5914      | BOP1 | block of proliferation 1 | -1.27 | -1.32 | -2.04 | -2.22 |
| NM_007161   | B2RP | block of proliferation 1 | -1.19 | -1.33 | -2.00 | -2.13 |
| NM_005091   | PG11RP | ribosomal protein D15 | -1.64 | -1.19 | -2.38 | -2.38 |
| NM_007221   | PMF1 | telomeric segregation increased 2-like 4 | -2.00 | -1.89 | -3.33 | -2.08 |
| AK025211    | ASAP | similar to rat nuclear ubiquitin conjugate kinase 2 | -1.67 | -0.88 | -2.44 | -2.33 |
| NM_009852   | PP155 | phosphatidylinositol (4,5) bisphosphate 5-phosphatase homolog, phosphatidylinositol 3-phosphate 5- | -1.47 | -1.04 | -2.63 | -2.22 |
| NM_000788   | DCK | deoxyxystidine kinase | -1.49 | -1.18 | -2.38 | -2.08 |
| AB023144    | SEZ6 | deformation-related homolog (mouse)-like | -2.38 | -1.35 | -2.17 | -2.00 |
| AF254866    | ZNF278 | zinc-finger protein 278 | -2.00 | -1.45 | -1.69 | -2.70 |
| NM_012686   | LOC50138 | carp-1 | -2.36 | -1.61 | -2.08 | -2.56 |
| NM_001916   | CYC1 | alpha-tubulin | -3.57 | -1.59 | -2.44 | -2.44 |
| NM_014037   | PRKKG2 | chromosome segregation 1-like | -3.57 | -1.75 | -1.79 | -2.17 |
| AP219203    | HCAP-G | chromosome condensation protein G | -2.70 | -1.49 | -1.69 | -2.78 |
| X89672      | ADAM1 | a disintegrin and metallopeptidase domain 3a (cytokeratin 1) | -2.04 | -0.94 | -2.15 | -2.00 |
| NM_010730   | KIF3 | KIF3 | -5.88 | -10.00 | -2.04 | -2.04 |
| NM_015974    | LOC35084 | lambda crystallin | -1.49 | -2.70 | -5.88 | -3.13 |
| NM_003544    | H4FI | histone family, member 1 | -1.61 | -2.08 | -2.70 | -2.56 |
| NM_017541    | CRTGS | crystallin, gamma S | -2.50 | -1.69 | -2.08 | -1.89 |
| NM_002851    | PXK48 | phosphatidylinositol 4-kinase, catalytic, beta polypeptide | -2.17 | -2.17 | -2.50 | -2.63 |
| NM_003684    | SAP3 | s15-associated polypeptide, 3KD | -2.08 | -2.08 | -2.08 | -2.17 |
| NM_013357    | PVRB | purine-rich element binding protein G | -3.33 | -3.23 | -2.08 | -2.08 |
| MJ7191    | Sp3 | Sp3 transcription factor | -2.50 | -1.92 | -3.23 | -4.00 |
| NM_007474    | CB114A | cholinergic receptor, nicotinic, alpha polypeptide 4 | -1.22 | -1.16 | -1.79 | -2.04 |
| NM_001268    | APA51 | adaptor-related protein complex 3, sigma 1 subunit | -1.11 | -1.43 | -1.79 | -2.08 |
| NM_004966    | HNRFP | heterogeneous nuclear ribonucleoprotein F | -1.18 | -1.18 | -2.38 | -2.22 |
| AK025496    | MMS19L | similar to rat nuclear ubiquitin conjugate kinase 2 | -1.32 | -1.39 | -1.96 | -2.27 |
| NM_004581    | KCTQ2 | potassium voltage-gated channel, KQT-like subfamily, member 2 | -1.14 | -1.18 | -2.17 | -1.32 |
| NM_005410    | SEPP1 | selenoprotein P, plasma, 1 | -1.09 | -2.00 | -2.17 | -1.54 |
| NM_000071    | CBS | CBS | -1.00 | -2.08 | -3.13 | -1.43 |
### Table 1.  
Continued

| Acc. Number | Gene Name | Description | 6 h | 8 h | 12 h | 24 h |
|-------------|-----------|-------------|-----|-----|------|------|
| NM_001759  | CCND2     | cyclin D2   | -2.70 | -2.02 |
| NM_002826  | QCN6      | quiescin Q6 | -2.22 | -1.20 | -2.94 | -1.00 |
| NM_012090  | MACF1     | microtubule-actin crosslinking factor 1 | -2.08 | -1.27 | -2.27 | -1.12 |
| NM_019079  | FLJ10884  | hypothetical protein | -2.38 | -1.11 | -2.00 | -1.11 |
| XT9924     | ZNF266    | zinc finger protein 266 | -3.85 | -1.35 | -1.72 | -1.03 |
| NM_064672  | TXNIP     | thioredoxin interacting protein | -2.33 | -1.23 | -3.03 | -1.16 |
| AK003630   | ETS1      | v-ets erythroblastosis virus E26 oncogene homolog 1 (avian) | -2.70 | -1.33 | -2.08 | -0.97 |
| NM_005622  | SAH       | SA hypertension-associated homolog (rat) | -2.63 | -1.00 | -2.33 | -1.20 |
| D26070     | ITPR1     | inositol 1,4,5-triphosphate receptor, type 1 | -3.33 | -1.15 | -2.08 | -0.81 |
| NM_003836  | DLK1      | delta-like 1 homolog (Drosophila) | -2.08 | -1.35 | -2.33 | -0.93 |
| NM_004852  | BNP3      | BCL2/adenovirus E1B 19K interacting protein 3 | -2.44 | -1.19 | -1.96 | -1.35 |
| AF274863   | SEC31B-1  | secretory pathway component Sec31B-1 | -2.00 | -1.33 | -1.54 | -1.59 |
| NM_003225  | TFF1      | trefoil factor 1 (breast cancer, estrogen-inducible sequence expressed in) | -4.17 | -1.27 | -1.23 | -1.20 |
| NM_000996  | CP        | ceruloplasmin (ferroxidase) | -3.85 | -1.69 | -1.89 | -0.81 |
| NM_001815  | SERPIND1  | serine (or cysteine) proteinase inhibitor, clade D (heparin cofactor), member 1 | -3.33 | -1.41 | -2.94 | -0.98 |
| NM_015863  | ZIM2      | zinc finger, imprunted 2 | -2.22 | -1.35 | -1.72 | -1.14 |
| AF227924   | SIGLEC9   | sialic acid binding Ig-like lectin 9 | -2.00 | -1.23 | -1.18 | -0.93 |
| NM_004821  | HAND1     | heart and neural crest derivatives expressed 1 | -2.44 | -1.75 | -1.16 | -0.93 |
| NM_005004  | BCA1      | branched chain amiotransferase 1, cytosolic | -2.56 | -1.72 | -1.23 | -1.03 |
| NM_002421  | MMP1      | matrix metalloproteinase 1 (interstitial collagene) | -2.24 | -0.75 | -1.47 | -1.00 |
| NM_002083  | GPX2      | glutathione peroxidase 2 (gastrointestinal) | -2.56 | -0.89 | -1.35 | -0.88 |
| AB033041   | VANGL2    | van gogh-like 2 (Drosophila) | -2.38 | -1.01 | -1.14 | -0.96 |
| NM_016073  | CGI-142   | CGI-142 | -2.56 | -0.94 | -1.28 | -0.89 |
| NM_003918  | GIY2      | glycogenin 2 | -7.14 | -1.28 | -1.37 | -0.89 |
| NM_006608  | ALEX1     | ALEX1 protein | -1.92 | -2.56 | -1.37 | -0.98 |
| NM_007123  | USH2A     | usher syndrome 2A (autosomal recessive, mild) | -2.86 | -2.50 | -1.89 | -0.93 |
| NM_007186  | CD8A      | CD8 antigen, alpha polypeptide (p32) | -2.00 | -2.08 | -1.59 | -1.25 |
| NM_032192  | TERT      | telomerase reverse transcriptase | -2.86 | -2.27 | -1.32 | -1.12 |
| NM_006633  | IQGAP2    | IQ motif containing GTPase activating protein 2 | -2.08 | -2.00 | -1.22 | -1.52 |
| US7784     | RDC1      | G protein-coupled receptor | -3.33 | -2.86 | -0.98 | -1.03 |
| NM_006408  | ACR2      | anterior gradient 2 homolog (Xenopus laevis) | -3.33 | -2.27 | -0.70 | -0.90 |
| NM_000558  | HBA1      | hemoglobin, alpha 1 | -2.70 | -2.13 | -2.08 | -0.76 |
| NM_004369  | COLIA3    | collagen, type VI, alpha 3 | -2.86 | -2.08 | -1.64 | -0.73 |
| NM_006994  | DCLK1     | deleted in liver cancer 1 | -2.33 | -2.56 | -1.18 | -1.10 |
| NM_01803   | CDW52     | CDW52 antigen (CAMP/MAPK-1 antigen) | -2.33 | -2.33 | -1.08 | -0.88 |
| NM_012113  | CA14      | carbonic anhydrase XIV | -2.27 | -2.08 | -1.32 | -1.04 |
| NM_004734  | DCAMKL1   | doublecortin and CaM kinase-like 1 | -3.85 | -3.03 | -1.27 | -0.98 |
| NM_005531  | IFH1      | interferon, gamma-inducible protein 16 | -7.69 | -5.56 | -1.37 | -0.76 |

Listed are genes that showed at least 2-fold changes in response to 5-FU treatment for at least two time points in HCT116 cells and these genes are sensitive to inhibition of p53 accumulation by CHX in the experiment shown in Fig. 3. Genes in bold are previously identified p53 targets.
A

Time 0 6 12 24 48 12 24 48 h

p53
Actin

5FU 5FU+CHX (+6h)

B

Apoptosis (%)

0 6 12 24 48

5FU 5FU+CHX (+6h)

C

5-FU: 0 2 4 6 8 12 24 0 6 8 10 12 24

fold induction (log2)

D

fold induction (log2)

0 8 12 24 0 8 12 24

5-FU 5-FU+CHX (+6h)
Fig. 6
A

Untreated  Control siRNA  PLK siRNA

- PLK
- NS

Untreated  Control siRNA  PTTG1 siRNA

- PTTG1
- NS

B

Untreated

Control siRNA

PLK siRNA

PTTG1 siRNA

1%  8%

23%  19%

C

Apoptosis induction

Control siRNA  PLK siRNA  PTTG1 siRNA

0  0.5  1  1.5  2  2.5
p53-regulated transcriptional program associated with genotoxic stress-induced apoptosis
Patricia S Kho, Zhen Wang, Li Zhuang, Yuqing Li, Joo-lin Chew, Huck-Hui Ng, Edison T Liu and Qiang Yu

J. Biol. Chem. published online March 11, 2004

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