Identification of Myc-mediated Death Response Pathways by Microarray Analysis*

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To understand the mechanisms of Myc-mediated apoptosis induced by DNA damage, we have characterized the death kinetics of three Rat-1 fibroblast cell lines that either overexpress Myc or lack Myc and their parental wild-type cells following exposure to the DNA-damaging agent VP-16, and we monitored the changes in gene expression using microarray. We have identified three groups of genes whose expressions are distinctly regulated during this process. One cluster (Cluster A) revealed a VP-16-dependent but Myc-independent induction of a set of genes that is not linked to the apoptotic response. Two other gene clusters, however, were associated with VP-16-induced apoptosis. Cluster B, which includes p53-responsive genes, was associated with the temporal onset of apoptosis but accounted for only the basal apoptosis. However, Cluster C, which includes c-jun, was highly regulated by Myc and appeared to be critical to mounting the maximal apoptotic response in Myc-expressing cells. Furthermore, the Myc level dropped sharply following VP-16 exposure, which varied inversely with the induction of Cluster C genes, suggesting Myc normally represses their transcription. Thus, we have proposed that removal of Myc-mediated repression of apoptotic signals, combined with Myc-associated acceleration of the p53 responsive pathway, results in complete and rapid cell death following DNA damage.

The proto-oncogene Myc has been shown to play a pivotal role in proliferation, differentiation, and apoptosis, and its abnormal expression has been associated with many human cancers. Myc exerts its biological effects as a transcription factor that not only promotes cell proliferation but also is required for efficient induction of apoptosis. However, Myc target genes have not been well defined. In particular, few Myc target genes have been associated with its pro-apoptotic function (1–3).

One possibility is that Myc promotes cell death by sensitizing cells to other apoptotic stimuli, rather than by acting as a direct death effector (4). Indeed, Myc expression has been shown to sensitize cells to a variety of apoptotic insults such as serum or growth factor deprivation (1, 2), nutrient privation (2), hypoxia (5), or response to DNA-damaging agents (6, 7). Despite a large body of literature, the mechanism by which Myc exerts its apoptotic effect remains elusive. In particular, little is known about the mechanism by which Myc mediates DNA damage-induced apoptosis. Recently, putative Myc target genes p19 ARF, cyclin A (7) have been shown to be specific for mediating the apoptotic function of Myc, thus providing direct molecular links between Myc and the induction of apoptosis.

Gene expression is required for transmitting DNA damage signals to apoptotic signaling machinery. Among the best characterized transcriptional programs activated by DNA damage is p53-dependent transactivation of its target genes such as p21, gadd45, and Bax, leading to cell growth arrest or apoptosis, depending on the cellular context. p53 has been implicated as a target of Myc regulation (9, 10), and execution of a full Myc-mediated apoptotic response requires p53 (6, 11, 12). The requirement of p53 for Myc-mediated apoptosis has been recently linked to p19 ARF, which negatively regulates the inhibitory MDM2 pathway, thus preventing MDM2-induced p53 degradation (8). However, the exact contribution of p53 to Myc-mediated apoptosis remains to be established. Other transcriptional programs independent of p53 response are also implicated in the DNA damage-induced apoptotic pathway (13, 14). The complexity of these results suggests that a genome-wide survey for Myc-mediated gene expression under apoptotic conditions might allow the unbiased identification of Myc target genes responsible for its pro-apoptotic function.

In this study, we describe the identification of Myc-mediated transcriptional programs in DNA damage-induced apoptosis. We have used three isogenic Rat-1 fibroblast cell lines that either overexpress Myc (Rat1-myc) or lack Myc (myc-null), as well as their parental cells (Rat1), to systematically compare gene expression profiles with cDNA microarray. Our study identified certain gene clusters that are most linked to Myc-mediated apoptosis.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—Myc wild-type fibroblast cells TGR-1 (Rat1) and the Myc−/− derivative (myc-null) were from John Sedivy (Brown University, Providence, RI) and have been described (15). The Rat1 cells overexpressing Myc (Rat1-myc) were obtained from C. V. Ng (The Johns Hopkins University, Baltimore, MD) and have been described (16, 17). VP-16 was purchased from Sigma and SB203580 from Calbiochem.

cDNA Arrays, Probes, and Hybridization—Construction of the microarray containing 6,500 rat cDNA clones selected from the Rat Gene Index of the Institute for Genomic Research was as described (18). cDNA probes were synthesized from amplified RNA using a modified method of Eberwine et al. (19) and have been described. The cDNA probes were made from 3 μg of amplified RNA with SuperScript II RT (Invitrogen) to incorporate Cy3 or Cy5 dye (Amersham Biosciences). Labeled Cy3 and Cy5 cDNA probes were cleaned up using a Microcon column (Millipore, Bedford, MA) and were combined, along with 2 μl of

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The abbreviations used are: ARF, alternative reading frame; RT, reverse transcription; PCNA, proliferating cell nuclear antigen; MAPR, mitogen-activated protein kinase; AEBSF, 4-(2-aminothyl)benzenesulfonyl fluoride.

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cot-1 DNA, 2 μl of poly(A), and added H2O, to a volume of 20 μl. After heating the samples at 95 °C for 5 min, cDNA probes were added to 20 μl of 2× hybridization buffer (50% formamide, 10× SSC, 0.2% SDS) and centrifuged at 13,000 rpm for 10 min. For hybridization, array slides were first prehybridized with 5× SSC, 0.1% SDS, and 1% bovine serum albumin for 1 h, rinsed with H2O for 2 min, and dehydrated in isopropl alcohol for 2 min. 40 μl of cDNA probe were added to the slides and incubated overnight at 42 °C. The slides were then washed sequentially in 2× SSC, 0.1% SDS; 1× SSC, 0.1% SDS; 0.2× SSC; and 0.05× SSC before being scanned for image analysis using Genepix 3.0 software (Axon Instruments, Inc., Foster City, CA). To ensure the reliability of data, samples from each time point were also reciprocally labeled in all hybridization experiments. Microarray slides were scanned for image analysis using Genepix 3.0 software (Axon Instruments, Inc.).

Data Normalization, Filtering, and Cluster Analysis—Total intensity normalization was used to correct bias caused by systematic differences in labeling and detection efficiencies for the fluorescence labels. A normalization factor was calculated to rescale each gene in the array so that the median fluorescence ratio of all spots was 1.0. The data were then filtered so that only spots with intensities two times greater than background were used in the analysis. Changes of gene expression were presented as logarithmic ratios of fluorescence intensities. Data from reciprocal replicates were averaged after appropriate transformation. The log 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 1.5-fold change of expression value for at least two time points across all experiment conditions. A total of 985 of ~6,500 clones met the criteria and were further analyzed using clustering and display programs (rana.stanford.edu/software) developed by Eisen et al. (21).

RT-PCR—One microgram of total RNA from each sample was subjected to PCR with reverse transcription using the One Step RT-PCR kit (CLONTECH) according to the manufacturer’s protocol. Selected RNA species were amplified using the following primers: c-Jun, sense, 5'-acctgtgagaatgcc-3' and antisense, 5'-agagggtctgagactcaaac-3'; Fra-2, sense, 5'-actgctgtgcacagcagc-3' and antisense, 5'-gaagccacagcacagc-3'; IEX-1, sense, 5'-gtaactgcaagatactcacc-3' and antisense, 5'-atcagcacttcacgc-3'; and Gadd45, sense, 5'-gagacacatatgctcc-3' and antisense, 5'-ctgagaactgccctgc-3'. Amplification of the β-actin fragment was used as a PCR and gel-loading control. PCR was carried out for 20–30 cycles, with each cycle consisting of a denaturing step for 1 min at 94 °C, an annealing step for 2 min at 58 °C, and a polymerization step for 2 min at 72 °C. The PCR product was separated on a 1.0% agarose gel containing ethidium bromide and photographed under ultraviolet light.

Western Blot Analysis—Cell lysates were lysed with cell lysis buffer (0.3% Nonidet P-40, 1 μM 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 centrifuged at 12,000 g for 15 min at 4 °C. Protein concentration was quantified (Bio-Rad), and protein samples (100 μg) were separated by SDS-PAGE and transferred onto immobilon membranes (Millipore, Bedford, MA). To study the effect of SB203580 on c-Jun expression, cells were treated with 50 μM SB203580 for 1 h prior to VP-16 treatment, and c-Jun proteins were identified using anti-Jun antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Reactive bands were visualized using the enhanced chemiluminescence detection system (PerkinElmer Life Sciences).

RESULTS

Cell Death Kinetics Induced by VP-16 Correlate with Myc Status of Cells—We first assessed the effect of Myc on the cell death kinetics of Rat-1 cells in response to DNA damage. Rat-1 cells overexpressing Myc (Rat1-myc), lacking Myc (myc-null), and their parental TGR-1 cells (Rat1) were treated with 20 μM VP-16, a DNA-damaging agent that induces topoisomerase II (22) and causes double strand DNA breaks. The apoptotic Rat-1 cells became detached (Ref. 7 and Fig. 1A), and cell viability was determined by a trypan blue exclusion assay. As shown in Fig. 1, about 5 h after VP-16 treatment the Rat1-myc cells began to undergo apoptosis. In contrast, little apoptosis was observed in Rat1 and myc-null cells (Fig. 1A). To obtain detailed cell death kinetics for each cell line, cells were treated with VP-16 and monitored for up to 48 h. As shown in Fig. 1B, Rat1-myc cells underwent rapid apoptosis. Approximately 25% of the cells became apoptotic after 4 h of drug treatment, and the majority of cells became apoptotic after 12 h. By contrast, induction of apoptosis was delayed in Rat1 and markedly attenuated in myc-null cells. In Rat1 cells, 25% apoptosis was observed around 8 h after drug treatment, and most of the cells became apoptotic after 24 h, whereas the myc-null cells did not undergo detectable apoptotic response until 48 h after drug treatment. Moreover, a maximum of 25% apoptosis in myc-null cells was observed even 72 h after drug treatment (data not shown). We used this end point of a 25% apoptotic population as a target for our experiments. This observation indicated that Myc potentiated DNA damage-induced apoptosis: the apoptotic process was not just delayed but also impaired in the absence of Myc. Our observation is consistent with a recent study by Adachi et al. (7) demonstrating that the reduced susceptibility of myc-null Rat-1 cells to VP-16-induced apoptosis was not because of the slow growth rate of these cells. Thus, induction of apoptosis by VP-16 in Rat-1 cells appeared to depend upon the level of Myc. The differential apoptotic responses observed in these cells also suggested that VP-16 may activate two apoptotic signaling pathways in Rat1 fibroblast cells. One is a Myc-dependent signaling pathway that induced a strong and rapid apoptosis in Myc-expressing cells. The other appeared to be Myc-independent and accounted for the weak residual apoptotic response (25%) as was observed in myc-null cells.

Fig. 1. Myc expression potentiates DNA damage-induced apoptosis. A, morphological appearances of Rat1 fibroblast cells with constitutively overexpressed Myc (Rat1-myc), Rat-1 cells lacking Myc (myc-null), and their parental Rat-1 cells (Rat1) in response to VP-16 treatment. Cells treated with 20 μM VP-16 were examined microscopically 5 h after drug treatment. Apoptotic cells exhibited membrane blebbing and an increase in the number of round and/or detached cells. B, cell death kinetics of Rat-1-myc, Rat1, and myc-null cells following exposure to VP-16 treatment. Cells growing exponentially were subjected to 20 μM VP-16 treatment. At the indicated time points, cell viability was evaluated by a trypan blue exclusion assay, and the percentage of cell deaths was determined and plotted against time.
Transcriptional Responses to VP-16 Associated with Myc Expression—To investigate the transcriptional programs that underlie the differential cellular response as reported in Fig. 1, we made use of cDNA microarray technology to study the expression of 6,500 genes. Cells treated with VP-16 were harvested at different time points until a similar level of an apoptotic end point (25% cell death) had been reached in all three cell lines (Fig. 1B). Using RNA taken at the zero time point of untreated cells as reference, gene expression for each time point after drug treatment was monitored. Scatter plots in Fig. 2A show the effect of Myc on gene expression in response to VP-16 in the three cell lines. Four hours after drug treatment, greater alterations in gene expression were observed in myc-overexpressing Rat1-myc cells than Rat1 and myc-null cells. To ensure the reliability of data, samples from each time point were also reciprocally labeled in all hybridization experiments. The resulting two sets of duplicate data were plotted to assess assay reproducibility. As shown in Fig. 2B, a representative plot showed a correlation coefficient factor of 0.72 for total genes and 0.82 for genes with a change greater than 1.5-fold, respectively, indicating a good reproducibility between two duplicate assays. To compile a global view of gene expression response of the three cell lines following exposure to VP-16 treatment, genes that showed a greater than 1.5-fold difference after drug treatment for at least two time points in each cell line were selected. Based on these criteria, we found a total of 901 genes differentially expressed in VP-16-treated Rat1-myc cells, whereas 562 and 537 genes were differentially expressed in Rat1 and myc-null cells. To ensure the reliability of data, samples from each time point were also reciprocally labeled in all hybridization experiments. The resulting two sets of duplicate data were plotted to assess assay reproducibility. 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null cells, respectively (Fig. 2C). Noticeably, the number of genes induced by VP-16 diminished with decreasing levels of Myc. Compared with the 356 genes induced by VP-16 in Rat1-myc cells, there were 304 and 199 genes induced in wild-type Rat1 and myc-null cells, respectively (Fig. 2C). This observation indicates that Myc expression augments the transcriptional response to DNA damage caused by VP-16 treatment.

To visualize the time-dependent expression patterns that could potentially reveal the principles underlying the Myc-mediated cellular phenotype, we performed hierarchical clustering of the outlier genes as defined above. To further reduce random fluctuations of gene expression, we applied an additional selection for outlier genes, selecting those that vary in the same direction in two of the three Myc conditions. This criterion demands consistent directionality in the temporal expression profiles as related to the expression dose of Myc. Our analysis revealed three clusters of up-regulated genes that responded to VP-16 in a Myc-associated manner based on the magnitude of induction and the timing of the peak effect. The first cluster (Fig. 3A, Cluster A) represents a group of genes that is dramatically induced by VP-16 in magnitude and in time but appears to be independent of cellular Myc status. This group includes members of the Ras/MAPK pathway and suggests that these genes may be proximately induced by the DNA damaging agent.

The second group contains genes (Fig. 3A, Cluster B) associated with the timing of apoptosis but whose magnitude of induction by VP-16 was unrelated to Myc levels. Gene expression in this cluster showed a rapid increase within 4 h after VP-16 treatment in Rat1-Myc cells. In contrast, a similar level of gene induction was not observed until 8 h after drug treatment in Rat1 cells and 48 h in myc-null cells, respectively (Fig. 3B). Of interest, this cluster contains genes involved in cellular stress and growth arrest, and many of them have been previously recognized as p53 target genes. For example, gadd45, gadd153, cyclin G1, PCNA, BTG2, fas, and MCI-1 have been well-known p53 target genes that are involved in DNA damage-induced growth arrest or apoptosis. RT-PCR analysis of Gadd45 confirmed the expression pattern observed in the array data (Fig. 3C). This observation suggested that p53 responsive genes are associated with the physical onset of VP-16-induced apoptosis and that their trigger for temporal expression is

FIG. 3. Gene expression profiles in Clusters A and B. A, cluster images showing genes whose levels of expression in response to VP-16 are independent of Myc. Genes were clustered hierarchically based on the similarity of their expression profiles. Cluster A represents genes that show a rapid induction in all three cell lines. Cluster B represents genes whose induction by VP-16 did not require Myc but can be accelerated by Myc expression. Genes encoding known p53 targets are marked in red. B, graphs showing the differential kinetics of gene induction of these cells in Cluster B. Each time point represents an averaged expression ratio of these genes in Cluster B. C, RT-PCR analysis of Gadd45.
linked to Myc status. An alternative explanation is that these genes are associated with the residual 25% apoptosis that can be induced in the absence of Myc but that this portion of the apoptotic response is an important initiator for a wider Myc-associated death response. This cluster also contains other genes involved in stress response (hyaluronan synthase, histone H3, Hsp70, and translation initiation factors) and growth arrest (nerve growth factor inducible protein) that have not previously been associated with p53 and may represent markers of the apoptotic process. Other well studied p53 target genes involved in mediating cell cycle arrest (p21) and apoptosis (Bax) were not present in our microarray and therefore could not be assessed.

The most striking cluster, (Fig. 4A, Cluster C) represents genes whose optimum induction after DNA damage is highly dependent on the Myc status of the cells. Gene expression in this cluster was strongly induced by VP-16 in Rat1-myc cells, leading to a mean 5-fold induction in magnitude within 4 h after drug treatment. In contrast, induction of these genes was significantly reduced in Rat1 cells, and little response was observed in myc-null cells even up to 48 h (Fig. 4, A and B), coinciding with the impaired ability to undergo apoptosis in these cells. Genes in this cluster include c-jun, fra-2, IEX-1, raf-1, and ID-1 that have been previously reported to be involved in the regulation of apoptosis (23–29). Among them, induction of c-Jun, Fra-2, NRP/B, and ID-1 by VP-16 appeared to be completely dependent on the Myc status because there was no transcriptional activation in myc-null cells. These observations were confirmed by RT-PCR analysis (Fig. 4C). Taken together, this suggests that Cluster C genes are the primary Myc-regulated genes associated with the optimal induction of apoptosis after VP-16 exposure. Of note is that this response is not required for the 25% basal level of apoptosis at 48 h seen in myc-null cells. Therefore, expression of these genes during apoptosis may account for the full Myc-dependent apoptosis in Myc-expressing cells.

**c-Jun Expression Varies Inversely with Myc Expression Following VP-16 Treatment and Correlates with Myc-mediated Apoptosis**— Genes identified in Cluster C such as c-jun and IEX-1 have been implicated in both pro-survival and pro-apoptotic events (24, 25, 27). To determine the exact effects of these genes in Myc-mediated apoptosis, we examined whether changes in c-Jun expression correlate to Myc-mediated apoptosis. We took advantage of previous reports that activation of
p38 kinase results in c-Jun and c-Fos induction (30, 31) and that p38 kinase is implicated in Myc-mediated apoptosis putatively through Jun and Fos induction (32, 33). This was accomplished by testing the effect of SB203580, a p38 kinase inhibitor, on VP-16-induced c-Jun expression and its correlation to apoptosis. The level of c-Jun protein increased following VP-16 treatment (Fig. 3A), paralleling the increase in c-Jun mRNA from RT-PCR and array analysis. Pretreatment of Rat1-myc cells with SB203580 blocked the induction of c-Jun by VP-16 (Fig. 3A) and decreased VP-16-induced apoptosis in Myc-expressing cells (Fig. 3B). However, in myc-null cells where no detectable induction of c-Jun was seen after DNA damage (Fig. 4, A and C and data not shown), no inhibition of apoptosis was observed (Fig. 5B). These data suggest that c-Jun expression contributes to Myc-dependent apoptosis. Interestingly, we found that Myc protein expression dropped dramatically after VP-16 treatment (Fig. 5C), which was followed by an immediate induction in c-Jun expression. This finding suggests that genes shown in Cluster C may potentially be the targets of transcriptional repression by Myc. A drop in Myc during DNA damage-induced apoptosis removed the Myc-mediated gene repression, allowing activation of transcription of these genes.

Absence of VP-16-induced Transcriptional Down-regulation Specific to Myc-dependent Apoptosis—As noted before, we selected only those outlier genes that varied consistently with the dose of Myc expression and found consistent up-regulated genes with such an association. Our microarray data analysis, however, did not reveal genetic elements that were consistently down-regulated by VP-16 yet displayed a Myc- or apoptosis-dependent manner. For example, though a set of 89 genes was found to be down-regulated by VP-16 in Rat-myc cells (data not shown), repression of this gene set was not observed to be Myc dose-dependent in Rat-1 and myc-null cells and thus was considered to be irrelevant to Myc-dependent apoptosis. Therefore, though down-regulated genes may have a function in VP-16-induced apoptosis, neither the pathways nor the functions are consistent across different dosages of steady-state Myc expression.

DISCUSSION

In our previous study (18) as well as a study by others (34), microarray technology was used to identify Myc responsive genes associated with growth and proliferation. These observations showed that Myc induces gene cassettes associated with protein synthesis. One of the most intriguing aspects of Myc is its ability to potentiate the apoptotic effects of a wide range of cellular insults. In this research, we extended those studies to systematically examine the genes altered during apoptotic response to a DNA-damaging agent, VP-16, that may be differentially regulated by Myc. The substantial difference in apoptotic response in Rat1 fibroblast cell lines differing only in Myc status provided an ideal system to interrogate expression of genes associated with death pathways supported by Myc. Furthermore, our study design allows for subtraction of transcriptional “noise” due to clonal selection and clonal drift. In filtering outlier genes, we demanded that these outliers be associated with either a common phenotype (apoptosis) or with the gene dosage of Myc. Our array data analysis suggests that there are discernable Myc-dependent and Myc-independent apoptotic pathways that are transcriptionally regulated. Cluster A (Fig. 3A) represents general responsive genes induced by VP-16 regardless of cellular status of Myc and may represent genes whose promoters are sensitive to DNA damage or cellular stress. Included in this cluster are Ha-Ras and mitogen-activated kinase kinase (MKK) that often mediate the earlier signaling events in response to a stress stimulus. Thus, though the downstream function of their gene products cannot be discerned by our experiments, this gene list may represent those that act as indicators of cellular stress.

Induction of p53 Responsive Genes and Myc-associated Apoptosis—Analysis of our data shows that members of the p53 pathway (Cluster B) are induced in the apoptotic process. It has been reported that Rat1 fibroblast cells null for myc displayed an impaired p53 response to VP-16 compared with its parental cells (7), possibly due to defective expression of the Myc target gene, ARF (8). Though it has been proposed that p53 is required for Myc-induced apoptosis (4, 12), our results suggest a more complicated picture. Our time course analysis of gene expression suggests that though the induction of p53 responsive genes is delayed in the absence of steady-state Myc expression, the p53 pathway alone may not be fully responsible for the Myc-mediated death response. This is because only a maximum of 25% apoptosis could be reached in myc-null cells even though a comparable level of p53 response was obtained at 48 h. In support of this, overexpression of p53 in myc-null Rat-1 cells did not fully restore DNA damage-induced apoptosis (7). Moreover, p53 null mouse embryo fibroblasts are not totally resistant to Myc-induced apoptosis (8). Thus, the p53-mediated response may be mainly responsible for the initiation of cellular apoptosis or a necessary step for sensitizing the apoptotic process. Our results suggest a further model: the p53 pathway triggers a basal level of apoptosis after VP-16 in the absence of Myc (i.e., the residual 25% apoptosis at 48 h in myc-null cells) but requires Myc to engage additional
death effector pathways necessary for a maximal apoptotic response.

**Myc-dependent Gene Expression Implicated in the Regulation of Apoptosis**—The most striking finding of our data analysis is the identification of a class of genes that responded to VP-16 that are highly dependent on the cellular Myc status. These genes were induced mainly in Myc-expressing cells undergoing optimal apoptotic response to VP-16. Thus, we have proposed that Cluster C genes may represent those Myc-regulated t-elements that can collaborate with p53 responsive triggers to engage a strong cellular apoptosis.

We found that the Myc protein level dropped sharply in response to VP-16 treatment. This effect could be due to the transcriptional down-regulation of Myc as reported by others (36–38). The immediate increase of gene transcription seen in Cluster C following the drop in Myc protein indicates that Myc normally represses the transcription of these genes. Increasing evidence suggests that transcriptional repression is necessary for Myc-induced transformation and proliferation (39). Myc has been found to repress the transcription of a growing number of genes involved in cell cycle control or growth arrest, such as p21 (40,41), p27 (37,39,41), p15 (42), gadd45 (43), gadd153 (43,44), and gas1 (45) in order to promote cell proliferation. More recently, Myc-mediated transrepression and not transactivation has been shown to be directly responsible for Myc-induced apoptosis, because deletion of the MB II domain of Myc that mediates a transrepression function drastically impaired the ability of Myc to drive apoptosis (20). In further support of this, our data analysis revealed no cluster of VP-16 downregulated genes correlating with Myc-dependent apoptosis. Thus, this study is in agreement with the previous report by Conzen et al. (20) and further suggests that selective transcription of death-associated genes due to the removal of Myc-mediated transrepression may be critical for Myc-induced apoptosis.

The results presented in this study, together with those by others, suggest a working model for Myc in DNA damage-induced apoptosis (Fig. 6). This model reflects a balance between the ability of Myc to drive transformation and apoptosis. In the normal situation, Myc selectively represses the transcription of apoptotic signals in favor of cell proliferation. In the presence of DNA damage or other apoptotic stimuli, a drop in Myc expression will elevate those otherwise repressed pro-apoptotic genes contributing to Myc-dependent cell death. Induction of p53 responsive transcripts accounts for the baseline of Myc-independent apoptosis, but the onset for this pathway appears to be accelerated in the presence of Myc, which synergizes the Myc-specific pathway and results in an optimal apoptotic response.

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