c-Myc Sensitization to Oxygen Deprivation-induced Cell Death Is Dependent on Bax/Bak, but Is Independent of p53 and Hypoxia-inducible Factor-1*

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Deregulated expression of c-Myc can sensitize cells to a variety of death stimuli, including loss of growth factors and oxygen. In this study, we examined whether rodent fibroblasts that conditionally express c-Myc undergo a similar mechanism of cell death in response to serum or oxygen deprivation. Our results demonstrate that murine embryonic fibroblasts from bax−/−bax−/− mice that conditionally express c-Myc did not die in response to either oxygen or serum deprivation. Fibroblasts from p53−/− mice that conditionally express c-Myc died in response to oxygen (but not serum) deprivation. The inability of p53 to regulate oxygen deprivation-induced cell death was due to the lack of induction of p53 target genes Puma, Noxa, and Pten. In contrast, serum deprivation transcriptionally induced Puma and Pten in cells that conditionally express c-Myc. The failure of p53 to regulate oxygen deprivation-induced cell death led us to hypothesize whether hypoxia-inducible factor (HIF) might be a critical regulator of cell death during oxygen deprivation. Fibroblasts from HIF-1β−/− cells that conditionally express c-Myc were not able to transcriptionally activate HIF during oxygen deprivation. These cells died in response to oxygen deprivation. Thus, oxygen deprivation-induced cell death in fibroblasts with deregulated expression of c-Myc is independent of p53 or HIF-1 status, but is dependent on the Bcl-2 family member Bax or Bak to initiate mitochondrial dependent cell death.

The proto-oncogene c-Myc is a transcription factor that forms a heterodimer with Max and activates genes involved in proliferation (1). However, cells expressing c-Myc rapidly undergo cell death under conditions in which survival factors are limiting (reviewed in Ref. 2). The molecular machinery that regulates c-Myc-induced cell death is distinct and independent from proliferation because activation of the molecular machinery mediating cell cycle progression is not required for c-Myc-induced cell death (3, 4). c-Myc itself does not induce cell death, but acts to sensitize cells to other death stimuli. c-Myc expression has been shown to sensitize cells to a wide variety of death stimuli, including serum and growth factor deprivation (5, 6), glucose deprivation (7), oxygen deprivation (8, 9), virus infection (10), tumor necrosis factor (11), interferons (12), CD95/Fas (13), and p53-dependent response to genotoxic damage (6). The fact that c-Myc can sensitize to so many different triggers of cell death suggests an action at some common point in the death machinery. Previous studies have demonstrated that Myc-induced sensitization to death stimuli is mediated through changes in outer mitochondrial membrane permeabilization, resulting in the release of cytochrome c from the mitochondria to the cytosol (14, 15).

Recent studies have indicated that key regulators of outer mitochondrial membrane permeabilization are the Bcl-2 family proteins (reviewed in Refs. 16 and 17). Bcl-2 and Bcl-xL prevent outer mitochondrial membrane permeabilization in response to apoptotic signals, whereas the Bcl-2 family proteins Bax and Bak promote it (18–22). The overexpression of Bcl-2 and Bcl-xL can prevent Myc-overexpressing cells from serum or oxygen deprivation (8, 23–26). Primary cells isolated from mice lacking both Bax and Bak are resistant to cell death signals that cause outer mitochondrial membrane permeabilization, such as staurosporine, UV radiation, etoposide, thapsigargin, and serum and oxygen deprivation (27–29). Bcl-2 family proteins are also key downstream targets of the transcription factor p53 or survival-signaling pathways such as that initiated by insulin-like growth factor-1. Insulin-like growth factor-1 or loss of p53 can inhibit growth factor or serum deprivation-induced death in cells with deregulated c-Myc (4, 30–31).

The ability of Myc to concurrently induce proliferation and cell death has led to the proposal that death due to inadequate survival factors provides a mechanism to guard against the appearance of neoplastic clones that can lead to unstrained cell growth. Tumors with deregulated Myc expression require a continuous supply of survival factors or the acquisition of mutations such as in the p53 pathway or overexpression of Bcl-2 or Bcl-xL (32–34). Two survival factors that are likely to be limiting to induce cell death in tumors...
with deregulated c-Myc are growth factors and oxygen. Presently, it remains unknown whether loss of either oxygen or growth factors elicits a similar death pathway in cells with deregulated c-Myc expression. In this study, we investigated the role of p53 and Bcl-2 proteins in regulating oxygen deprivation-induced cell death in fibroblasts that express a conditionally active c-Myc and compared these genetic manipulations in the same cells exposed to serum deprivation.

MATERIALS AND METHODS

Cell Culture—Rat1a fibroblasts and murine embryonic fibroblasts (MEFs) \(^3\) were cultured to 30–40% confluence in Dulbecco’s modified essential medium supplemented with 25 mM HEPES, 1 mM pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (Invitrogen). Primary MEFs were generated from wild-type, p53−/−, or bax−/−/bak−/− embryos. Rat1a fibroblasts or MEFs stably expressing a DNA encoding human c-Myc fused at its C terminus to the hormone binding domain of 4-hydroxymethylxanthen (4-OHT)-responsive mutant murine estrogen receptor (MycER) were established by retroviral infection as previously described (35). Rat1a fibroblasts containing MycER and expressing Bcl-xL or dominant-negative p53 (GSE56) were established by retroviral infection as described previously (36, 37). GSE56 in pLXSII was a gift of Andrei Gudkov. Primary MEFs were generated from HIF-1α−/− and HIF-1α+/− embryos. After 30 min with buffer containing NADH then reduces tetrazolium (yellow) to formazan (red), which was detected at 490 nm. Lactate dehydrogenase release is expressed as the percentage of the lactate dehydrogenase measured in the medium over the total lactate dehydrogenase released after treating with Triton X-100.

Measurement of Caspase-9 Activation—Rat1a/MycER cells were plated on 60-mm culture dishes at 30% confluence and exposed to experimental conditions. Briefly, 500 μl of medium was removed, and the remaining cells were lysed by adding the same volume of 0.5% Triton X-100. After 30 min, 500 μl of the lysate was removed. The samples were incubated for 30 min with buffer containing NADH, lactate, and tetrazolium. Lactate dehydrogenase converts lactate to pyruvate, generating NADH. The NADH then reduces triazolium (yellow) to formazan (red), which was detected at 490 nm. Lactate dehydrogenase release is expressed as the percentage of the lactate dehydrogenase measured in the medium over the total lactate dehydrogenase released after treating with Triton X-100.

RESULTS

c-Myc Sensitizes Rat1a Fibroblasts to Serum or Oxygen Deprivation-induced Cell Death—Deregulated c-Myc expression in Rat1a fibroblasts without serum or oxygen has been demonstrated to cause death (6, 8–9). Rat1a fibroblasts were stably transfected with a fusion protein containing a modified hormone-binding domain of the murine estrogen receptor and the full-length human c-Myc protein (Rat1a/MycER cells) (35). This c-Myc chimeric protein can be activated by the addition of 100 nm) to cell cultures. Rat1a/MycER cells were cultured at low confluence (30–40%) under normal oxygen conditions (21% O2) in medium containing 10% fetal bovine serum (FBS) and 25 mM HEPES to buffer any changes in pH. Rat1a/MycER cells were incubated with or without OHT for 16 h. Subsequently, Rat1a/MycER cells were deprived of either serum or oxygen for 16 and 24 h. Total cell death was measured by lactate dehydrogenase release. The addition of OHT accelerated cell death in these cells when deprived of either serum or oxygen (Fig. 1, A and B). In accordance with previous studies, our present data demonstrate that expression of c-Myc accelerates cell death following oxygen or serum deprivation.

c-Myc Sensitization to Serum (but Not Oxygen) Deprivation-induced Cell Death Requires p53—The cell death activity of p53 has been suggested to be responsible for suppression of Myc-mediated tumorigenesis (38). Studies have also demonstrated that c-Myc-mediated cell death is dependent on p53 in rodent fibroblasts (4). We examined the role of p53 in our Rat1a/MycER cells by stably expressing a dominant-negative mutant of p53 (DNp53) (39). In control experiments, Rat1a/MycER cells containing DNp53 were markedly protected against daunorubicin-induced cell death by a trimmer of a hypoxic response element (HRE-luciferase) and 50 ng of Rev30a luciferase driven by the herpes simplex virus thymidine kinase (TK) promoter (TK-luciferase; Promega). HRE-luciferase reporter constructs consisted of a primed mer containing 18 bp of sequence from the phosphoglycerate kinase promoter. Luciferase assays were performed using the Dual Luciferase assay system (Promega). Values are reported as a ratio of HRE-luciferase to TK-luciferase.

RNA Isolation and Real-time Reverse Transcription-PCR—Total RNA was isolated from cells grown in monolayers using a modified guanidine isothiocyanate protocol (TRIZol reagent, Invitrogen) according to the manufacturer’s protocol. The expression of p21, Puma, Noxa, and Pten was determined by real-time quantitative PCR using the ABI PRISM 7000 sequence detector system (PerkinElmer Life Sciences) and the TaqMan gold reverse transcription-PCR kit (Applied Biosystems). Murine primers and probes were selected based on nucleotide sequences downloaded from the NCBI Data Bank and designed by Primer Express Software 1.0 (Applied Biosystems); Puma sense, 5'-TGCACT-GATGGAGATACGGACCT-3'; Puma antisense, 5'-ACCATGACTCTTCAGCCCTC-3'; Puma TaqMan probe, 5'-CTGCGCCTCCGAGAACCCAGG-3'; Pten sense, 5'-GCCAAGCTGCCAGACAT-3'; Pten antisense, 5'-TCCATCCTCTGTATATCCTCTTTT-3'; Pten TaqMan probe, 5'-ACGACCCATCATCAAGAGATGCTGACGA-3'; P21 sense, 5'-CTGTTCGCGCAGGAGGCC-3'; P21 antisense, 5'-CCGCAACTGCTCA-3'; P21 TaqMan probe, 5'-TGTGCCCCTGTCTCCTGTCTGTC-3'; Noxa sense, 5'-CTGAGGCACTTCGACGAGCC-3'; Noxa antisense, 5'-CCTCCTAGTCTCCGAGCCAC-3'; Noxa TaqMan probe, 5'-ATGAGGGCCCAAGCAGCCACCC-3'. The TaqMan probe consists of an oligonucleotide with a 5’-reporter dye (6-carboxyfluorescein) and a 3’-quencher dye (6-carboxytetramethylrhodamine). The reaction mixture was prepared according to the manufacturer’s protocol (Applied Biosystems). All samples were read in duplicate, and values were normalized for base-line expression and for expression of glyceraldehyde-3-phosphate dehydrogenase (internal reference).

Immunoblotting—The primary antibodies used for immunoblotting were anti-p53 monoclonal antibody (Ab-1, Oncogene Research) at a concentration of 4.0 μg/ml and anti-a-nitulin antibody (B-11-2 (Sigma) at 5.0 μg/ml. The secondary antibody used was horse-radish peroxidase-linked anti-mouse IgG (H + L antibody) (Cell Signaling) at a concentration of 1 μg/ml. Cell lysates were prepared using cell lysis buffer (New England Biolabs Inc.) supplemented with 1 mM phenylmethylsulfonyl fluoride.

The abbreviations used are: MEFs, murine embryonic fibroblasts; OHT, 4-hydroxymethylxanthen; HIF-1, hypoxia-inducible factor-1; HRE, hypoxic response element; TK, thymidine kinase; FBS, fetal bovine serum; DNp53, dominant-negative p53; WT, wild-type.

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bicin-induced cell death (Fig. 2A). Rat1a/MycER cells containing DNp53 and incubated with OHT were protected from serum (but not oxygen) deprivation-induced cell death (Fig. 2, B and C). We further confirmed our findings by expressing the conditional OHT-dependent c-Myc protein in MEFs from wild-type (p53+/+/MycER MEFs) and p53-null (p53−/−/MycER MEFs) animals. Both p53+/+/MycER and p53−/−/MycER cells were incubated with OHT for 16 h and subsequently deprived of either serum or oxygen for 24 h. p53−/−/MycER cells incubated with OHT were protected from serum deprivation-induced cell death (Fig. 3). In contrast, p53−/−/MycER cells incubated with OHT were not protected from oxygen deprivation-induced cell death. Collectively these results indicate that p53 is required for serum (but not oxygen) deprivation-induced cell death in rodent fibroblasts that conditionally express c-Myc.

Bcl-2 Proteins Regulate c-Myc Sensitization to Serum or Oxygen Deprivation-induced Cell Death—Previous studies have indicated that the ability of Bcl-2 or Bcl-xL to inhibit cell death is through prevention of Bax or Bak from causing outer mitochondrial membrane permeabilization (reviewed in Ref. 39). To test whether Bax or Bak is required for oxygen or serum deprivation-induced cell death in cells overexpressing c-Myc, the conditional OHT-dependent c-Myc protein was expressed in MEFs from wild-type (WT/MycER MEFs) and Bax- and Bak-null animals (bax−/−/bak−/−/MycER MEFs). Both WT/MycER and bax−/−/bak−/−/MycER MEFs were incubated with OHT for 16 h and subsequently deprived of either serum or oxygen for 24 h. The bax−/−/bak−/−/MycER cells incubated with OHT were protected from both serum and oxygen deprivation-induced cell death (Fig. 5). These results indicate that both serum and oxygen for 16 and 24 h (Fig. 4, A and B). Recent studies have shown that Bcl-xL prevents either serum or oxygen deprivation-induced cell death (Fig. 2A). Rat1a/MycER cells containing DNp53 and incubated with OHT were protected from serum (but not oxygen) deprivation-induced cell death (Fig. 2, B and C). We further confirmed our findings by expressing the conditional OHT-dependent c-Myc protein in MEFs from wild-type (p53+/+/MycER MEFs) and p53-null (p53−/−/MycER MEFs) animals. Both p53+/+/MycER and p53−/−/MycER cells were incubated with OHT for 16 h and subsequently deprived of either serum or oxygen for 24 h. p53−/−/MycER cells incubated with OHT were protected from serum deprivation-induced cell death (Fig. 3). In contrast, p53−/−/MycER cells incubated with OHT were not protected from oxygen deprivation-induced cell death. Collectively these results indicate that p53 is required for serum (but not oxygen) deprivation-induced cell death in rodent fibroblasts that conditionally express c-Myc.

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oxygen deprivation-induced cell death pathways converge on Bax or Bak to cause outer mitochondrial membrane permeabilization.

**Bcl-xL (but Not p53) Regulates Oxygen Deprivation-induced Cytochrome c Release and Caspase-9 Activation**

We further investigated the role of p53 and Bcl-xL in regulating cell death by examining cytochrome c release and caspase-9 activation. Cells release cytochrome c early in the course of cell death due to loss of outer mitochondrial membrane integrity. Cytochrome c in the cytosol binds to Apaf-1 (apoptotic protease-activating factor-1), which then undergoes a conformational change that allows the cleavage and activation of caspase-9 (40–43). Rat1a/MycER cells were incubated with OHT for 16 h, subsequently deprived of either serum or oxygen for 16 h, and analyzed for cytochrome c release and caspase-9 activation. It is important to note that there was minimal cell death at 16 h in response to either death stimulus (see Fig. 1). The addition of OHT resulted in increased levels of cytochrome c release and caspase-9 activation in Rat1a/MycER cells following oxygen or serum deprivation compared with cells that did not receive OHT (Fig. 6). Rat1a/MycER cells overexpressing Bcl-xL did not release cytochrome c or activate caspase-9 upon deprivation of serum or oxygen in the presence of OHT for 16 h, subsequently deprived of either serum or oxygen for 16 h, and analyzed for cytochrome c release and caspase-9 activation. It is important to note that there was minimal cell death at 16 h in response to either death stimulus (see Fig. 1). The addition of OHT resulted in increased levels of cytochrome c release and caspase-9 activation in Rat1a/MycER cells following oxygen or serum deprivation compared with cells that did not receive OHT (Fig. 6). Rat1a/MycER cells overexpressing Bcl-xL did not release cytochrome c or activate caspase-9 upon deprivation of serum or oxygen in the presence of OHT (Fig. 6). In contrast, Rat1a/MycER cells containing DNp53 in the presence of OHT released cytochrome c and activated caspase-9 in response to oxygen (but not serum) deprivation (Fig. 6). Thus, Bcl-xL prevents both the cytochrome c release and caspase-9 activation in Rat1a cells conditionally expressing c-Myc upon serum or oxygen deprivation. The loss of p53 function prevents only serum deprivation-induced cell death in Rat1a fibroblasts conditionally expressing c-Myc at a level upstream of cytochrome c release and caspase-9 activation.

**Oxygen Deprivation Fails to Activate p53 Target Genes in Cells with Deregulated c-Myc Expression**

To further understand why p53 is required only under conditions of serum (but not oxygen) deprivation, we examined p53 protein levels and p53 transcriptionally regulated genes p21, Puma, Noxa, and Pten in WT/MycER MEFs in the absence of either serum or oxygen. Puma and Noxa are BH3 proteins that can activate Bax or Bak and have been implicated in p53-dependent cell death (44). Pten is a negative regulator of the phosphatidylinositol 3-kinase/Akt-dependent cellular survival signal and has been shown to be required for p53-mediated cell death in immortalized MEFs (45). It has been proposed that c-Myc favors the initiation of cell death by inhibiting the expression of the cyclin-dependent kinase inhibitor p21 and allowing the p53-dependent expression of cell death genes such as Puma (46). WT/MycER MEFs were incubated with OHT for 16 h and deprived of either serum or oxygen for 6 h (prior to any cell death), and the levels of p53 protein and p53 target genes were assessed. WT/MycER MEFs treated with OHT stabilized p53 protein levels under normal oxygen conditions as well as serum or oxygen deprivation (Fig. 7A). However, the p53 target genes...

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**Figure 3.** Wild-type or p53–/– MEFs that express a conditional OHT-dependent c-Myc protein were incubated under normal oxygen conditions with 10% FBS in the presence of OHT (100 nM) for 16 h. Subsequently, cell death was measured in cells deprived of either oxygen or serum for 24 h. Data are expressed as means ± S.E. from four independent experiments.

**Figure 4.** Rat1a/MycER cells overexpressing Bcl-xL were incubated under normal oxygen conditions with 10% FBS in the presence of OHT (100 nM) for 16 h. Subsequently, cell death was measured in Rat1a/MycER cells deprived of either oxygen (A) or serum (B) for 16 and 24 h. Data are expressed as means ± S.E. from four independent experiments.

**Figure 5.** Wild-type or bax–/– bak–/– fibroblasts that express a conditional OHT-dependent c-Myc protein were incubated under normal oxygen conditions with 10% FBS in the presence of OHT (100 nM) for 16 h. Subsequently, cell death was measured in cells deprived of either oxygen or serum for 24 h. Data are expressed as means ± S.E. from four independent experiments.
**c-Myc Sensitization to Oxygen Deprivation-induced Cell Death**

**FIG. 6.** Rat1a/MycER cells stably expressing Bcl-xL or DNp53 were incubated under normal oxygen conditions with 10% FBS in the presence of OHT (100 mM) for 16 h. Subsequently, cytochrome c release (A) or caspase-9 activation (B and C) was measured in cells deprived of either oxygen or serum for 16 h. Cytochrome c immunostaining and caspase-9 activation were measured in three independent experiments, and data are expressed as means ± S.E.

Puma and Pten were expressed only under normal oxygen conditions or serum deprivation in the presence of OHT (Fig. 7B). Noxa mRNA levels were suppressed under either serum or oxygen deprivation, whereas p21 levels were not significantly affected under either condition (Fig. 6B). These results indicate that p53 is not transcriptionally active upon oxygen deprivation in cells with deregulated c-Myc expression.

**DISCUSSION**

The oncoprotein c-Myc is a potent inducer of cell proliferation (1). However, neoplastic cells such as those containing c-Myc are limited in growth because of vascular deficiency or defective microcirculation. This results in an inadequate supply of oxygen, glucose, and growth factors to the tumor mass. Cells expressing c-Myc rapidly undergo death when deprived of these critical survival factors. For the tumor to grow bigger than 1 mm³, cells must induce the formation of new blood vessels (angiogenesis) and adapt to be able to survive in a state where survival factors are limiting (49). The adaptation of cells for survival is linked to escaping normal cell death mechanisms initiated during loss of survival factors. In this study, we examined cell death mechanisms initiated during loss of two critical survival factors (oxygen and serum) in rodent fibroblasts expressing conditionally active c-Myc. In accord with previous findings, the expression of c-Myc itself did not yield cell death, but rather resulted in sensitization to either oxygen or serum deprivation.

The ability of c-Myc to sensitize cells to serum or oxygen deprivation implies a common point in the death machinery where diverse cell death signals converge. The release of cytochrome c has been proposed as a point in the death machinery where diverse cell death signals could converge (14). In this study, we have demonstrated that Rat1a cells expressing c-Myc released cytochrome c prior to cell death in response to serum or oxygen deprivation. The overexpression of Bcl-xL prevented death in Rat1a cells with deregulated Myc expression in response to either oxygen or serum deprivation. Bcl-xL also prevented the release of cytochrome c from the mitochondria and the consequential activation of caspase-9 during oxygen or serum deprivation. The release of cytochrome c is mediated through activation of Bax or Bak (reviewed in Ref. 39). The loss of Bax and Bak is sufficient to render primary MEFs resistant to serum or oxygen deprivation-induced cell death (28, 29). The anti-apoptotic property of Bcl-xL is attributed to preventing the activation of Bax or Bak. Bax has been shown as a direct gene target of Myc (50). Bax activity within the mitochondria of apoptotic cells is stimulated in the presence of c-Myc (51).

Furthermore, the loss of Bax alone markedly reduces growth factor deprivation-induced cell death in primary murine pre-B cells or in rodent fibroblasts with deregulated c-Myc expression (52, 53). In this work, we have extended these previous findings...
by demonstrating that primary MEFs from mice deficient in the bax and bak genes expressing c-Myc were completely resistant upon serum or oxygen deprivation. These observations indicate that both serum and oxygen deprivation in Myc-expressing fibroblasts utilize the pro-apoptotic Bcl-2 family members as a common point in the apoptotic machinery to initiate mitochondrial dependent cell death.

Although serum or oxygen deprivation signals cell death ultimately through Bcl-2 family members regulating cytochrome c release, our current data suggest that both serum and oxygen deprivation in Myc-expressing fibroblasts utilize different upstream pathways that modify the Bcl-2 family members in rodent fibroblasts with deregulated Myc expression. This is supported by the observation that the loss of p53 function impaired only serum (but not oxygen) deprivation-induced cell death in fibroblasts with deregulated c-Myc expression. Previous studies have indicated that p53-dependent and p53-independent pathways regulate death in cells with deregulated c-Myc expression during serum deprivation. The p53-independent pro-apoptotic action occurs through unknown effectors that cause cytochrome c release through the outer mitochondria membrane, which is prevented by the overexpression of Bcl-xL (14). The p53-dependent pathway involves the transcriptional induction of the tumor suppressor ARF by c-Myc, which inhibits the p53 antagonist Mdm-2 and triggers accumulation of p53 (54). Consistent with these previous findings, we have reported here that Myc did stabilize p53 protein levels under normal oxygen conditions as well as upon serum or oxygen deprivation. Myc selectively inhibits p53 from activating p21 transcription and initiates the transcription of pro-death proteins such as Bax, Noxa, and Puma (55–58). Furthermore, Pten, a negative regulator of Akt, is transcriptionally induced by p53 (45). Akt provides a survival signal by preventing pro-apoptotic Bcl-2 family members from initiating mitochondrial dependent cell death (15). Although

![Image](image-url)
and co-workers (63), who demonstrated that loss of p53 function in primary baby mouse kidney epithelial cells containing the E1A oncogene is sufficient for transformation, but not tumorigenesis. The further loss of Bax and Bak in these transformed cells is required to generate tumors in nude mice. We speculate that the lack of oxygen encountered in growing tumors is able to induce death in baby mouse kidney epithelial cells that have only p53 function loss, thereby limiting tumor growth. The additional loss of Bax and Bak would be able to overcome oxygen deprivation-induced cell death, thus allowing transformed cells to become a solid tumor.

The inability of p53 to regulate cell death during oxygen deprivation led us to examine whether HIF-1 might be required for cell death. HIF-1 is a master transcriptional regulator of genes during oxygen deprivation (48). Previous studies have suggested both pro- and anti-apoptotic roles for HIF-1. The pro-apoptotic role of HIF-1 during oxygen deprivation is attributed to the transcriptional induction of the Bcl-2 family member BNIP3 (47). However, in this study, we found cell death to be HIF-1-independent in MEFs that ectopically express c-Myc. At present, it remains unknown which BH3 proteins are likely to regulate oxygen deprivation-induced death in cells with deregulated Myc expression. Our results do suggest that oxygen deprivation is likely to have a unique upstream cell death pathway that ultimately utilizes pro-apoptotic Bcl-2 family members to initiate mitochondrial dependent cell death.

The precise mechanisms by which p53 induces cell death have not been definitively determined, the loss of Pten or the loss the BH3-only proteins Puma and Noxa does impair p53-mediated cell death (45, 59–62). There is not likely to be a single gene that is the principal mediator of the p53 apoptotic signal, and it seems likely that this response represents the activation of several death genes. In this study, we found that Puma and Pten were transcriptionally up-regulated during serum deprivation in cells with deregulated Myc expression, whereas p21 transcription was unaltered. The up-regulation of Puma and Pten mRNAs during Myc expression coupled with the loss of phosphatidylinositol 3-kinase activity during serum deprivation likely contributes to the rapid cell death upon serum deprivation.

Oxygen deprivation failed to initiate Puma, Noxa, or Pten transcription in cells with deregulated Myc expression. These data indicate that the inability to activate p53 death genes during oxygen deprivation results in a p53-independent death pathway in cells with deregulated expression of c-Myc. These findings are consistent with previous data reported by Giaccia and co-workers (63), who demonstrated that, despite the induction of p53 protein levels during oxygen deprivation in human cancer cells, there is no induction of p53 target genes. The failure of p53 to activate genes was attributed to the inability of p53 to interact with the transcriptional activator p300 during oxygen deprivation. Interestingly, the loss of a p53 homolog (cep-1) in Caenorhabditis elegans is not required for oxygen deprivation-induced cell death, but is required for DNA damage-induced cell death (64). Our current data might also provide a molecular explanation for a recent study by Degenhardt et al. (65), who demonstrated that loss of p53 function in primary baby mouse kidney epithelial cells containing the E1A oncogene is sufficient for transformation, but not tumorigenesis.

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