Specific Requirement for Bax, Not Bak, in Myc-induced Apoptosis and Tumor Suppression in Vivo*

Received for publication, December 22, 2005 Published, JBC Papers in Press, February 7, 2006, DOI 10.1074/jbc.M513655200

Tobias B. Dansen, Jonathan Whitfield, Fanya Rostker, Lamorna Brown-Swigart, and Gerard I. Evan

From the Cancer Research Institute and Department of Cellular and Molecular Pharmacology, Comprehensive Cancer Center, University of California at San Francisco, San Francisco, California 94143-0875

Bax and Bak comprise the mitochondrial gateway for apoptosis induced by diverse stimuli. Loss of both bax and bak is necessary to block cell death induced by such stimuli, indicating a great degree of functional overlap between Bax and Bak. Apoptosis is the major intrinsic pathway that limits the oncogenic potential of Myc. Using a switchable mouse model of Myc-induced apoptosis in pancreatic ß cells, we have shown that Myc induces apoptosis in vivo exclusively through Bax but not Bak. Furthermore, blockade of Myc-induced apoptosis by the inactivation of Bax, but not Bak, eliminates all restraints to the oncogenic potential of Myc, allowing the rapid and synchronous progression of invasive, angiogenic tumors.

Bax and Bak are multidomain Bcl-2 family members that function as an obligate gateway for the activation of apoptosis via the mitochondrial and endoplasmic reticular pathways (1–3). In contrast to the BH3-only proteins, which function as transducers of the apoptotic signal upstream of the mitochondria, Bax and Bak also contain BH1 and -2 domains and function at the mitochondrial outer membrane, presumably as pore-forming complexes, to release holocytochrome c in response to diverse afferent stimuli (4). Consequently, cells lacking both Bax and Bak exhibit marked resistance to diverse pro-apoptotic insults, such as DNA damaging agents and activation of death receptor pathways. In such instances, loss of either Bax or Bak alone exerts no measurable protective effect (3). Furthermore, mice deleted for either bax or bak alone are viable, indicating either no defects (in the case of Bax) or defects in only a few discrete lineages (in the case of Bak) (5, 6). In contrast, mice lacking both Bax and Bak die in early embryogenesis due to the failure of apoptosis in multiple developing tissues (6). Taken together, these observations indicate that Bax and Bak exhibit extensive functional degeneracy.

Myc is a pleiotropic transcription factor implicated in diverse cellular and tissue processes ranging from cell growth, cell cycle regulation, differentiation, metabolism, apoptosis, and angiogenesis to invasion (7–10). When active, Myc appears to potentiate all of these diverse biological programs simultaneously, with the outcome depending upon the circumstance, cell type, and local somatic environment of the cell. Myc is deregulated and/or overexpressed in many cancers (11). However, recent studies using switchable transgenic animal models have shown that the activation of Myc alone is insufficient to drive tumorigenesis in great part because of the overwhelming tumor-suppressive influence of apoptosis. Consistent with this, secondary oncogenic lesions that suppress apoptosis and/or enhance proliferative rate over that of apoptosis are sufficient to expose the oncogenic potential of Myc (12–15). The pivotal role in tumor suppression played by the innate apoptotic activity of Myc is evident in the well described pIns-MycERTAM model in which a switchable form of the Myc oncoprotein, MycER TAM, is transgenically targeted to pancreatic ß cells via the insulin promoter. Acute activation of MycER TAM triggers abrupt entry of ß cells into cycle, but this is accompanied by overwhelming apoptosis that rapidly leads to ß cell involution and onset of diabetes. In contrast, when Myc-induced apoptosis in ß cells is blocked by co-expression of the apoptosis suppressor Bcl-xL, rapid and synchronous tumorigenesis occurs in all islets (14). Not only does this demonstrate that apoptosis is a critical restraint to the otherwise potent and pleiotropic oncogenic potential of Myc, but the dramatic anti-apoptotic influence of Bcl-xL also indicates that Myc-induced apoptosis proceeds exclusively through the mitochondrial Bax/Bak pathway, presumably through the activation of specific BH3 proteins that are antagonized by the anti-apoptotic Bcl-2/Bcl-xL proteins. Consistent with this, in vitro experiments indicate that Bax is important in mediating Myc-induced apoptosis (at least in fibroblasts) and that Bax may contribute to limiting the oncogenic potential of Myc (16). By contrast, the role of Bak in such tumor-suppressive processes remains undefined.

The advantage of the acutely switchable pIns-MycERTAM model is that it allows direct observation of the acute consequences of Myc activation for proliferation and apoptosis in an orthotopic in vivo setting as well as how such acute effects elaborate into tumorigenesis. We have therefore used pIns-MycERTAM mice to assess the relative roles of Bax and Bak in mediating Myc-induced apoptosis and the suppression of Myc-induced tumorigenesis. We show that Myc-induced apoptosis is mediated solely through Bax and not Bak. Moreover, blocking apoptosis by deleting bax unleashes the full potential of Myc to drive the rapid and synchronous formation of angiogenic, invasive ß cell tumors.

MATERIALS AND METHODS

Manipulation of Transgenic Animals—Both pIns-MycERTAM and pIns-MycER TAM;RIP7-Bcl-xL mice have been described previously (14). bax−/− mice were purchased from Jackson Laboratories (Bar Harbor, ME) (stock number 002994), whereas bax−/− mice (5, 6) were provided by Dr. Craig Thompson (Department of Molecular Genetics and Microbiology, State University of New York at Stony Brook, NY). Strains were crossed to generate requisite genotypes, which were then verified by PCR analysis on genomic DNA isolated from tail snips. Tamoxifen (1 mg/20 g bodyweight, dissolved in peanut oil) (Sigma) or vehicle control (peanut oil) (Sigma) administration was initiated at 6–8 weeks of age and repeated for up to 12 consecutive days by daily intraperitoneal injection. For each bax or bak genotype, 12 mice were treated: 3 pIns-MycERTAM and three non-transgenic mice with tamoxifen and 3 pIns-MycERTAM and three non-transgenic mice with oil control. Blood glucose (using Accu-Chek strips, Roche Applied Science) and
animal weight were monitored before treatment and after 6 and 12 days of tamoxifen administration. All mouse experiments were approved by the University of California at San Francisco Institutional Animal Care and Use Committee.

Harvesting of Pancreata—Mice were injected with 150 μl of 10 mg/ml BrdUrd (Sigma) solution dissolved in saline 3.5 h before sacrifice, euthanized with CO2 and perfused through the heart with 6 ml of phosphate-buffered saline followed by 6 ml of zinc-buffered formaldehyde (Anatech, Battle Creek, MI). Pancreata were harvested and fixed overnight at 4 °C, dehydrated, processed, embedded in paraffin wax, and sectioned.

Histochemistry—Immunohistological detection of insulin, glucagon, PECAM-1 (CD31), as well as hematoxylin and eosin staining, were as described previously (14). BrdUrd incorporation and TUNEL2 staining were assayed using, respectively, a Roche Applied Science BrdUrd labeling and detection kit and the Chemicon (Temecula, CA) Apoptag peroxidase kit, according to the manufacturers’ instructions, with the modification that sections were then counterstained with Gill’s hematoxylin.

Confocal Laser Scanning Microscopy—Primary antibodies against insulin, Bak (Cell Signaling, Beverly, MA; 1:50) or Bak (Cell Signaling; 1:200) were applied as described for insulin (14). Anti-guinea pig-Alexa488 or anti-rabbit-Alexa568 (Molecular Probes, Leiden, The Netherlands) were used as secondary antibodies, as appropriate. Sections were embedded in DAKO fluorescent mounting medium containing 1 mg/ml Hoechst 33258 to stain nuclear DNA. Images were taken with a Zeiss LSM Meta confocal microscope.

Quantification of Apoptosis and Proliferation—Mice were treated with a single injection of tamoxifen and sacrificed 32 h later. TUNEL and BrdUrd staining on pancreas sections was done as above. Apoptotic cells and BrdUrd-positive cells were counted in at least 6 islets/mouse, with a minimum total of 500 β cells. Negligible apoptosis was observed in non-β cells using a double stain for TUNEL and insulin (data not shown).

RESULTS

Both Bax and Bak Are Expressed in Pancreatic β Cells—To assess the relative influence of Bax and Bak on Myc-induced tumorigenesis in pancreatic β cells in vivo, we first established that both Bax and Bak proteins are expressed in pancreatic β cells. Pancreata were harvested from bax+/−;bakin−/− mice, fixed, paraffin-embedded, sectioned, and stained with antibodies specific for either Bax or Bak proteins. Abundant and comparable staining of both Bax and Bak was observed in β cells of bax+/−;bakin−/− mice (Fig. 1). Bax was expressed at similar levels in both exocrine and endocrine tissue (Fig. 1A), whereas Bak expression was more prominent in the endocrine compartment (Fig. 1B). Analogous staining was performed on pancreas sections from either bax−/− or bak−/− animals, and this confirmed the specificity of the signal of each antibody (Fig. 1).

Absence of Bax, but Not Bak, Incapacitates Myc-induced β Cell Apoptosis and Allows Rapid β Cell Expansion—MycERTAM is functionally active only in the presence of its activating ligand 4-hydroxytamoxifen (4-OHT). MycERTAM was activated in mice in vivo by intraperitoneal administration of tamoxifen, which is then rapidly converted into 4-OHT by the liver. Acute activation of MycERTAM in pancreatic β cells induces both cell cycle entry but also overwhelming apoptosis leading to net β cell involution. As described previously, sustained activation of MycERTAM for 12 days by daily tamoxifen injection induced complete and universal ablation of islet β cells (Fig. 2A) (14). To dissect out the respective roles of Bax and Bak in Myc-induced β cell apoptosis, switchable plox-MycERTAM transgenic mice were crossed into either heterozygous or homozygous bax or bak knock-out backgrounds. MycERTAM was then continuously activated for 12 days, pancreata harvested, and sections stained for insulin to identify β cells in the islets. β cells of animals lacking one or both copies of bax behaved exactly like wild-type β cells, undergoing rapid involution upon activation of Myc. In complete contrast, β cells lacking both copies of bax did not involute and, instead, underwent rapid, synchronous, and progressive expansion, similar to that observed in β cells expressing the apoptosis inhibitor Bcl-xL (14). β cells in animals lacking only one copy of bax behaved like wild types, undergoing rapid involution (Fig. 2A). Tamoxifen treatment of mice lacking the MycERT transgene were unaffected by tamoxifen irrespective of Bax or Bak status, neither involuting nor expanding (Fig. 2B).

To ascertain that β cell expansion in the absence of Bax is specifically because of a blockade of Myc-induced apoptosis, we used TUNEL staining to identify apoptotic cells in pancreas sections from mice in which MycERTAM had been continuously activated for 12 days. In each case, sections were co-stained for glucagon to mark the positions of hypo- and hyperplastic islets; glucagon is a marker for insulin-negative islet α cells that do not express MycERTAM and, consequently, do not undergo apoptosis upon tamoxifen treatment. TUNEL-positive cells were evident in the shrunken involuted islets in all tamoxifen-treated plox-MycERTAM mice in which at least one copy of bax was present (Fig. 3, A

2The abbreviation used is: TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling.
Myc Kills through Bax but Not Bak

FIGURE 2. Loss of Bax prevents Myc-induced ablation of β cells. Mice were treated daily for 12 days with either tamoxifen or vehicle control (peanut oil). Paraffin sections of pancreata were stained for insulin (diaminobenzidine; brown) and counterstained with hematoxylin. Whole sections stained for insulin (diaminobenzidine; brown) and nut oil). Paraffin sections of pancreata were treated with vehicle or tamoxifen. Arrows indicate some islet remnants.

A. pancreatic islets, stained strongly for insulin, are clearly visible in tamoxifen-treated bax+/+;bax+/+, bax−/−;bax−/−, and bax−/−;bax−/− non-transgenic animals. B. Vehicle-treated bax+/−;bax+/− plns-MycERTAM pancreata show a similar pattern of insulin-stained islets (far left panel). However, after 12 days of sustained MycERTAM activity, islets are largely absent in bax+/−;bax+/− plns-MycERTAM mice. The same is observed for islets in bax−/−;bax+/− plns-MycERTAM, bax+/−;bax−/− plns-MycERTAM, and bax−/−;bax−/− plns-MycERTAM mice, which are also extensively involuted following sustained MycERTAM activation. In contrast, islets in bax−/−;bax+/− plns-MycERTAM do not involute following activation of MycERTAM and instead undergo rapid Myc-driven hyperplasia. L, lymph node. Arrows indicate some islet remnants.

and B). In such animals, islet remnants consisted predominantly of glucagon-positive α cells. In normal islets, glucagon-expressing cells represent no more than 10% of the cells in each islet, with the bulk of the remaining cells being β cells. Thus, the great preponderance of α cells in the remnants of involuted islets attests to the extensive apoptotic attrition that Myc induces within the β cell compartment. In contrast, islets from homozygous bax−/− deficient animals were all markedly hyperplastic with no discernible TUNEL-positive staining. Moreover, such hyperplastic bax−/− islets exhibited a vast excess of β cells over α cells, indicative of selective β cell hyperplasia (Fig. 3B). Of note, islet remnants were never observed in any of the bax−/− mice, indicating that none of the islets had involuted and, consequently, that the hyperplastic islets do not represent a select subset of β cells that expand after Myc activation. Hetero- or homozygous loss of bax exerted no detectable suppressive influence on Myc-induced apoptosis, consistent with the notion that Bak plays no significant role in Myc-dependent apoptosis. No TUNEL staining was evident in control tamoxifen-treated, non-transgenic pancreatic β cells of any genotype (Fig. 3A) nor in plns-MycERTAM animals of any bax or bax genotype treated with oil carrier instead of tamoxifen. Islets in such animals remained normal throughout (data not shown).

To exclude the possibility that tamoxifen treatment of bax−/− plns-MycERTAM mice somehow leads secondarily to loss of Bak in the islets, we examined Bak and Bak expression after 12 days of MycERTAM activation. β cells in hyperplastic islets of bax−/− plns-MycERTAM mice maintained Bak expression (compare Figs. 4A and 1B), confirming that β cells in bax−/− plns-MycERTAM islets fail to undergo apoptosis upon MycERTAM activation, despite the continued presence of Bak. Of note, the few β cells that remained in involuted bax−/− plns-MycERTAM islets showed clear evidence of Bak expression (Fig. 4B).

It nonetheless remained possible that Myc-induced apoptosis, although not completely blocked, was reduced in bax-deficient β cells, but that such partial protection was overwhelmed by 12 days of sustained Myc activation. We therefore also examined the extent of β cell apoptosis in mice in which MycERTAM had been activated for only 32 h. Once again, we discerned no suppression of apoptosis in animals lacking one or both copies of bax. By contrast, homozygous bax−/− deficient β cells exhibited complete resistance to Myc-induced apoptosis. Again, even a single copy of bax was enough to confer complete sensitivity to Myc-induced apoptosis in the absence of bak (Fig. 5). Proliferative rates upon activation of c-MycERTAM were not affected by Bak and Bak status, and the percentage of BrdUrd-positive cells was ~4% in all cases where the plns-MycERTAM transgene product had been activated (data not shown).

Sustained Activation of Myc in the Absence of Bax, but Not Bak, Leads to Rapid Onset of β Cell Tumorigenesis—As described previously, sus-
tained activation of MycERTAM in β cells co-expressing Bcl-xI leads to synchronous and progressive β cell expansion that is accompanied by lock-step co-expansion of the adjacent endothelial compartment (angiogenesis) together with local invasion of surrounding exocrine pancreatic tissue (14). Concomitantly, the affected β cells undergo partial loss of differentiation, down-regulate surface E-cadherin, and lose the intimate cell-cell contacts that characterize the normal islet. The rapidity and synchrony with which such neoplastic attributes arise indicate that these disparate pleiotropic changes are all instructed by the action of Myc, consistent with the normal physiological role of Myc as a

FIGURE 3. Apoptosis and glucagon staining of β cells after 12 days of sustained MycERTAM activation. Paraffin-embedded sections of pancreata from pIns-MycERTAM mice treated for 12 days with tamoxifen were stained for glucagon to identify α cells, whereas TUNEL labeling was used to identify apoptotic β cells. Dashed lines indicate the margins of islets or islet remnants. A, after 12 days of tamoxifen administration, islets appear normal in non-transgenic bax/bak wild-type mice, as indicated by the small relative proportion of α cells, which are largely confined to the islet periphery, and the lack of TUNEL-positive apoptotic cells. B, in pIns-MycERTAM transgenic bax−/−;bax−/− mice, however, only remnants of the islet remain, mostly comprising α cells, and most of the few remaining β cells are TUNEL-positive. Essentially the same is seen in islets from bax−/−;bax−/− pIns-MycERTAM, bax−/−;bax−/− pIns-MycERTAM, and bax−/−;bak−/− pIns-MycERTAM mice. In contrast, bax−/−;bak−/− pIns-MycERTAM islets are hyperplastic, have only a low proportion of glucagon-positive cells, and TUNEL-positive cells are largely absent.

Myc Kills through Bax but Not Bak

FIGURE 4. Bak expression is maintained in hyperplastic bax−/−;bax−/− pIns-MycERTAM islets following sustained Myc activation. False color multichannel confocal microscopy images show paraffin-embedded sections stained with anti-Bak (green), A) or anti-Bax (green, B) antibodies, exactly as for the untreated mice in Fig. 1. A, Bak expression is still clearly evident in hyperplastic islets from bax−/−;bax−/− pIns-MycERTAM animals, indicating that neither the expression nor stability of Bak is dependent upon the presence of Bax. B, Bak expression is also maintained in the few β cells remaining in involuted bax−/−;bax−/− pIns-MycERTAM islets after 12 days of MycERTAM activation.

FIGURE 5. Early stage Myc-induced apoptosis is not suppressed by the absence of Bak. To assess the influence of bax and bak status on β cell apoptosis at an early stage, prior to complete β cell ablation, the incidence of TUNEL-positive β cells was determined after only 32 h of MycERTAM activation when β cell involution was incomplete. Shown are TUNEL staining of representative islets from bax+/−, bax−/− pIns-MycERTAM (A) and bax−/−;bax−/− pIns-MycERTAM (B) mice. No TUNEL stain is evident in islets from non-transgenic animals (see also Fig. 3A) or in oil-treated transgenic pIns-MycERTAM controls (data not shown). C, quantitation of the percentage of apoptotic β cells per total islet in various genetic backgrounds shows that only loss of both bax copies blocks Myc-induced apoptosis. In all other genotypes, the extent of TUNEL-positive cells was ~7.5%. Error bars indicate standard deviations. Proliferative rates, as measured by BrdUrd incorporation, were not affected by Bax or Bak status (data not shown).
and thereby exert their neoplastic influence on neighboring cells and stroma. To determine whether the absence of Bax, but not Bak, confers a similar capacity on Myc to drive the formation of complex tumors, we examined the morphology, architecture, and dynamics of β cell hyperplasias arising from the activation of MycERTAM in β cells of bax-deficient mice and compared it to that in equivalently treated pIns-MycERTAM mice co-expressing Bcl-xL in their β cells (pIns-MycERTAM;RIP7-Bcl-xL).

Hematoxylin and eosin staining of hyperplastic islets in bax−/− mice in which MycERTAM had been activated for 12 days showed β cells to have enlarged nuclei containing dark chromatin, exactly as in analogously treated pIns-MycERTAM;RIP7-Bcl-xL animals (Fig. 6A, right panels). Multiple mitotic figures could be found in these islets. Systemic BrdUrd labeling (3.5 h intraperitoneal bolus prior to sacrifice) indicated that β cells remain in cycle after 12 days sustained Myc activity, exactly as in pIns-MycERTAM;RIP7-Bcl-xL mice. Indeed, even the proportions of BrdUrd-positive cells co-localized by exocrine tissue (inv). Such invasive edges are not observed in non-transgenic or vehicle-treated transgenic animals. β, bax−/−:bax−/− pIns-MycERTAM double transgenic mice also exhibited invasive edges that protrude into the exocrine tissue. In contrast, no BrdUrd-positive cells were visible in islets of pIns-MycERTAM transgenic mice in which MycERTAM never had been activated (Fig. 6A, left panels), irrespective of their Bax or Bcl-xL status. β cells in bax−/−:bax−/− pIns-MycERTAM mice showed dense insulin staining before MycERTAM activation. However, after 12 days of sustained Myc activity, insulin staining appeared patchy, and the mice exhibited acute diabetes, indicative of a lack of circulating insulin. Thus, as with pIns-MycERTAM;RIP7-Bcl-xL mice, even though β cell numbers progressively increased, the dominant differentiating influence of Myc was sufficient to curtail insulin production. By 12 days of Myc activation, bax−/−:bax−/− pIns-MycERTAM islets also exhibited invasive intrusions into the exocrine tissue, identical to those in pIns-MycERTAM;RIP7-Bcl-xL mice. Finally, Myc-induced β cell hyperplasias in bax−/−:bax−/− pIns-MycERTAM mice were highly vascularized as compared with non-tumorigenic islets from the same genetic background that were treated with the vehicle control rather than tamoxifen (Fig. 6B). This is consistent with earlier observations in pIns-MycERTAM;RIP7-Bcl-xL mice that Myc is potently angiogenic. Thus, by multiple independent parameters, Myc-induced tumors arising in bax-deficient animals resemble those in pIns-MycERTAM;RIP7-Bcl-xL mice in being dedifferentiated, invasive, and angiogenic. We conclude that loss of bax, but not bak, is sufficient not only to suppress Myc-induced apoptosis in vivo but also to unfetter the full oncogenic potential of Myc.

**DISCUSSION**

Previous *in vitro* studies have shown that Myc-dependent apoptosis in serum-deprived fibroblasts is dependent upon the expression of Bax but not Bak (16, 17). Our data confirm for the first time that the specific relationship between Myc and Bax holds in other cell types in a physiological setting *in vivo*. Of note, Myc does not induce expression of the bax gene but rather appears to trigger activation of the Bax protein (16, 10). Thus, its peculiarly private relationship with Bax implies the existence of some Bax-specific intermediate downstream of Myc, most likely...
in the maintenance of islet tumors that form in the bax<sup>−/−;</sup>bak<sup>+/+</sup> plus-MycER<sub>TAM</sub> mice at a later stage.

It remains to be seen whether Bak, in the absence of Bax, can mediate apoptosis in pancreatic β cells when it is induced by triggers other than Myc. It is known that β cell attrition can be induced in bax<sup>−/−;</sup>bak<sup>−/−;</sup>bak<sup>+/+</sup> mice, for example, by treatment with streptozotocin (26), although it has not been determined whether Bak is required to mediate this death. Ultimately, defining precisely when, and in response to what signals, Bax and Bak differentially contribute to apoptosis in tumors will be important in establishing if and when each can function to limit the emergence of tumors in vivo.

Acknowledgments—We are grateful to all members of the Evan laboratory for advice and comment.

REFERENCES

1. Oakes, S. A., Scorrano, L., Opferman, J. T., Bassik, M. C., Nishino, M., Pozzan, T., and Korsmeyer, S. J. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 105–110

2. Scorrano, L., Oakes, S. A., Opferman, J. T., Cheng, E. H., Sorcinelli, M. D., Pozzan, T., and Korsmeyer, S. J. (2003) Science 300, 135–139

3. Wei, M. C., Zong, W. X., Cheng, E. H., Lindsten, T., Panoutsokopoulou, V., Ross, A. J., Roth, K. A., MacGregor, G. R., Thompson, C. B., and Korsmeyer, S. J. (2001) Science 292, 727–730

4. Scorrano, L., and Korsmeyer, S. J. (2003) Biochem. Biophys. Res. Commun. 304, 437–444

5. Knudson, C. M., Tung, K. S., Tourtellotte, W. G., Brown, G. A., and Korsmeyer, S. J. (1995) Science 270, 96–99

6. Lindenst, T., Ross, A. J., King, A., Zong, W. X., Rathmell, J. C., Shios, H. A., Ulrich, E., Waymire, K. G., Mahar, P., Frauwhirt, K., Chen, Y., Wei, M., Eng, V. M., Adelman, D. M., Simon, M. C., Ma, A., Golden, J. A., Evan, G., Korsmeyer, S. J., MacGregor, G. R., and Thompson, C. B. (2000) Mol. Cell 6, 1389–1399

7. Dang, C. V. (1999) Mol. Cell. Biol. 19, 11–1

8. Lust, W., Leon, J., and Eilers, M. (2002) Biochim. Biophys. Acta 1602, 61–71

9. Pelengaris, S., Khan, M., and Evan, G. (2002) Nat. Rev. Cancer 2, 764–776

10. Soucie, E. L., Annis, M. G., Sedivy, J., Filмуs, J., Leber, B., Andrews, D. W., and Penn, L. Z. (2001) Mol. Cell. Biol. 21, 4725–4736

11. Nesbit, C. E., Tersak, J. M., and Prochownik, E. V. (1999) Oncogene 18, 3004–3016

12. Baudino, T. A., Maclean, K. H., Brennan, I., Parganas, E., Yang, C., Aslanian, A., Lees, J. A., Sherr, C. J., Rousell, M. F., and Cleveland, J. L. (2003) Mol. Cell 11, 905–914

13. Letal, A., Sorcinelli, M. D., Beard, C., and Korsmeyer, S. J. (2004) Cancer Cell 6, 241–249

14. Pelengaris, S., Khan, M., and Evan, G. I. (2002) Cell 109, 321–334

15. Schmitt, C. A., McCurrach, M. E., de Stanchina, E., Wallace-Brodeur, R. R., and Lowe, S. W. (1999) Genes Dev. 13, 2670–2677

16. Izbina, P., Hunt, A., Littlewood, T., Griffiths, B., Swigart, L. B., Korsmeyer, S., and Evan, G. I. (2000) Mol. Cell. Biol. 20, 6158–6169

17. Mitchell, K. O., Ricci, M. S., Miyashita, T., Dicker, D. T., Jin, Z., Reed, J. C., and El-Deiry, W. S. (2000) Cancer Res. 60, 6318–6325

18. Egle, A., Harris, A. W., Bouillet, P., and Cory, S. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6164–6169

19. Letal, A., Bassik, M. C., Walensky, L. D., Sorcinelli, M. D., Weiler, S., and Korsmeyer, S. J. (2002) Cancer Cell 2, 183–192

20. Hutcheson, J., Scarzizi, J. C., Bickel, E., Brown, N. J., Bouillet, P., Strasser, A., and Perlman, H. (2005) J. Exp. Med. 201, 1949–1960

21. Lindenhoin, L., Kringel, S., Braun, T., Borner, C., and Stein, R. (2005) Cell Death Differ. 12, 713–723

22. Chen, L., Willis, S. N., Wei, A., Smith, B. J., Fletcher, J. I., Hinds, M. G., Colman, P. M., Day, C. L., Adams, J. M., and Huang, D. C. (2005) Mol. Cell 17, 393–403

23. Willis, S. N., Chen, L., Dewson, G., Wei, A., Naik, E., Fletcher, J. I., Adams, J. M., and Huang, D. C. (2005) Genes Dev. 19, 1294–1305

24. Lowe, S. W., Cepero, E., and Evan, G. (2004) Nature 432, 307–315

25. Jamerson, M. H., Johnson, M. D., Korsmeyer, S. J., Furth, P. A., and Dickson, R. B. (2004) Br. J. Cancer 91, 1372–1379

26. Chi, M. M., Pingsterhaus, J., Carayannopoulos, M., and Moley, K. H. (2000) J. Biol. Chem. 275, 40252–40257

A. E. Hunt, A. J. Finch, B. Griffiths, L. Brown-Swigart, and G. I. Evan, submitted for publication.