Adenoviral Bid Overexpression Induces Caspase-dependent Cleavage of Truncated Bid and p53-independent Apoptosis in Human Non-small Cell Lung Cancers*

Received for publication, February 27, 2003, and in revised form, April 9, 2003 Published, JBC Papers in Press, April 10, 2003, DOI 10.1074/jbc.M302058200

Takuya Fukazawa, Barbara Walter, and Laurie B. Owen-Schaub‡

From the Department of Biomedical Sciences, University of California, Riverside, California 92521

Proapoptotic gene transfer to promote death or to augment killing by DNA-damaging agents represents a promising strategy for cancer therapy. We have constructed an adenoviral Tet-Off™ vector with tightly controlled expression of Bid (Ad-Bid) (Clontech, Palo Alto, CA). Using the non-small cell lung cancer cell lines H460, H358, and A549, low dose Ad-Bid was shown to induce high levels of full-length Bid as well as caspase-3 and -9 activity. Although only a small fraction of Bid was processed to truncated Bid (a step inhibited by benzyl-oxycarbonyl-Val-Ala-Asp-fluoromethyl ketone), Ad-Bid gene transfer resulted in mitochondrial changes consistent with apoptosis (mitochondrial depolarization, cytochrome c release), DNA fragmentation, and a dramatic loss of cell viability. The proapoptotic effects of Ad-Bid were independent of p53 status and were augmented markedly by caspase-8 activators such as the DNA-damaging agent cisplatin. When Ad-Bid and cisplatin were used together, chemosensitivity was restored in p53-null H358 cells, increasing death from 35% following treatment with cisplatin and Ad-LacZ to >90% death with Ad-Bid and cisplatin (Ad-Bid alone induced 50% cell death under these conditions). Ad-Bid can induce apoptosis in malignant cells and enhance chemosensitivity in the absence of p53, suggesting this approach as a potential cancer therapy.

Resistance to apoptosis has been proposed to create a platform that is necessary and sufficient for tumor formation (1). Sensitivity to apoptosis is known to be fundamental for theraeutic responses of malignant cells to cytotoxic immune cells, radiation, and chemotherapy (2). One of the most promising strategies in cancer gene therapy is the induction, augmentation, or restoration of apoptotic function through the transfer of proapoptotic genes. Apoptosis proceeds through two programs of caspase activation, known as the extrinsic and intrinsic pathways. The extrinsic pathway involves apoptosis-inducing extracellular receptors (such as tumor necrosis factor or Fas) that recruit and activate caspase-8 or -10 and, subsequently, effector caspases including caspase-3, -6, -7, and -9. The intrinsic apoptotic pathway is regulated by cytochrome c release (3) and typically is induced by stress, radiation, and chemotherapeutic drugs. Cytosolic cytochrome c induces caspase activation through the binding of apoptosis protease activation factor-1 and procaspase-9 to form the “apoptosome” leading to caspase-9 activation and caspase-3 processing. Although the extrinsic and intrinsic pathways can function independently, in most cells they are activated simultaneously and augmented by “cross-talk” of the reciprocal pathway.

The Bcl-2 family of apoptotic regulators is characterized by the presence of Bcl-2 homology (BH) domains and is subdivided into three groups comprising the antiapoptotic members Bcl-2 and Bcl-XL, the proapoptotic Bax-like proteins including Bak and Bax, and BH-3 domain-only proteins including Bid, Bik, Bim, and others. The BH-3 domain is a motif of ~16 residues forming an amphipathic helix required for dimerization among Bcl-2 family members and for death induction. Dimerization between Bcl-2 family members requires the BH-3 domain of one partner and a groove formed among BH-1, BH-2, and BH-3 domain helices of the other partner (4), although the role of dimerization in the regulation of mitochondrial membrane permeability and cytochrome c release remains incompletely resolved (5). Recent studies have revealed that BH-3-only domain proteins require at least one Bax-like partner to induce cell death (6, 7). Bid is one such proapoptotic protein that induces a conformational change in Bax or Bcl-2 resulting in a channel-forming complex in mitochondrial membranes (8–11). Bid is recognized as an intracellular link connecting the death receptor pathway and mitochondrial death machinery through caspase-8 activation (5). Full-length Bid, normally present in cytosol, is cleaved by activated caspase-8 to form tBid (carboxyl-terminal region of Bid), which translocates to the mitochondria causing cytochrome c release. Although tBid is a potent inducer of apoptosis, recent studies have shown that under some conditions full-length Bid can induce cytochrome c release in the absence of proteolytic cleavage (12).

Because Bid processing can link the extrinsic and intrinsic cell death pathways and amplify death receptor signaling, we have proposed Bid overexpression as a potential therapy for the management of cancer. In the present report, we show that a Tet-Off™ adenoviral vector expressing Bid (Ad-Bid) can result in high levels of Bid expression, tBid cleavage, and apoptosis in human NSCLC lines. The proapoptotic effects of Ad-Bid and tBid processing were viral dose-dependent and enhanced by chemotherapeutic agents such as cisplatin or Fas death receptor engagement. Ad-Bid could restore chemosensitivity to cisplatin in the absence of wild-type p53, suggesting Ad-Bid (alone

* This work was supported by Grant PO1-CA87778 from NCI, National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: University of California, Dept. of Biomedical Sciences, 1274 Webber Hall, Riverside, CA 92521; Tel.: 909-787-2583; Fax: 909-787-2438; E-mail: laurie.owenschaub@ucr.edu.

1 The abbreviations used are: BH, Bcl-2 homology; tBid, truncated Bid; Ad, adenovirus; Ad-Bid, adenoviral vector expressing Bid; Tet, tetracycline; NSCLC, non-small cell lung cancer(s); m.o.i., multiplicity of infection; PBS, phosphate-buffered saline; DOCA, 3,3’-dihexyloxacarbocyanine iodide; DFF, DNA fragmentation factor; fmk, fluoromethyl ketone; zVAD-fmk, benzylxycarbonyl-Val-Ala-Asp-fmk.
Ad-Bid Induces Apoptosis in NSCLC

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Culture Conditions**—The human NSCLC lines H460 (wild-type p53) and H358 (p53-null) were the generous gifts of Dr. A. Gazar, Northwestern Medical Center, Dallas, TX, and were grown in RPMI 1640 supplemented with 10% heat-activated fetal bovine serum. The human NSCLC line A549 (wild-type p53) was obtained from the American Type Culture Collection (ATCC) and was grown in Ham’s F-12 medium supplemented with 10% heat-activated fetal bovine serum. All cell lines were cultured in 10% CO2 at 37 °C.

**Antibodies**—Antibodies against human caspase-8, full-length Bid, DFF45, Bak, and Bam were purchased from Pharmingen. Antibodies against human cytochrome c and cytochrome c oxidase subunit 4 were purchased from Clontech. Anti-actin was purchased from Sigma. Secondary horseradish peroxidase-conjugated goat anti-rabbit antibody was obtained from Jackson Immunoresearch Laboratories (West Grove, PA). Antibody against Bid (p15) was purchased from BIOSOURCE International, Inc. (Camarillo, CA).

**Adenovirus Production**—Recombinant Tet-Off™ adenovirus containing human Bid was constructed according to the manufacturer’s protocol (Adeno-X™ Tet-Off™ expression system, Clontech). Human Bid cDNA was kindly provided by Dr. Gerhard Wagner (Harvard Medical School, Boston, MA). The construction and characterization of a recombinant adenovirus expressing human p53 (Ad-p53) and Escherichia coli β-galactosidase (Ad-LacZ) were described previously. Viruses were propagated in 293 packaging cells; tetracycline was present in the culture medium for Ad-Bid. Viral titer was determined by plaque assay. Cells were plated in 6-well plates at a titer of 1 × 10⁵ cells/well 1 day prior to virus infection. Unless otherwise indicated, cells were infected with adenovirus at a total m.o.i. of 5 plaque-forming units/cell. For Bid-infected cells, the ratio of the two vectors (Ad-Bid and Ad-Tet-Off™) was 1:1.

**Anti-Fas and Drug Treatment**—Anti-Fas (IPO-4, Kamiya, Seattle, WA) and cisplatin (Sigma) were used at a concentration of 1 and 2.5 μM, respectively. Sixteen hours after viral infection or anti-Fas treatment, adherent and floating cells were harvested, washed once in PBS, and fixed in 70% (v/v) ethanol overnight at 4 °C. Fixed cells were resuspended in PBS containing 0.2% Triton X-100 and 1 mg/ml RNase for 5 min and then stained with propidium iodide at 50 μg/ml to determine subdiploid DNA content using a FACScan (fluorescence-activated cell sorter scan). Doubles, cell debris, and fixation artifacts were gated out, and sub-G0/G1 DNA content was determined using CellQuest software, version 3.3.

**RESULTS**

**Ad-Bid Induces Caspase-mediated Processing of tBid in NSCLC—Using a Tet-Off™ adenoviral system to avoid the potential toxic effects of Ad-Bid on 293 packaging cells and to document that the NSCLC lines H460, A549, and H358 were transduced efficiently and produced high levels of Bid. As shown in Fig. 1A, full-length and truncated Bid were detectable in all three cell lines by Western blotting in the absence of tetracycline. Bid expression decreased to endogenous levels when tetracycline was present (compare +Tet to the mock controls) (Fig. 1A). Truncated Bid (15kD) was detectable only when Bid was expressed at relatively high levels (Fig. 1B). As shown in Fig. 1B (viral dose titration), tBid was reproducibly detected only at viral multiplicities of infection >5 despite the high levels of full-length Bid detectable at lower multiplicities of infection. The processing of Bid to tBid also correlated with the cleavage of DFF, substantiating apoptosis in infected cells (data not shown). DFF is a heterodimer composed of DFF40 (also known as CAD) and DFF45 (also known as ICAD). During apoptosis, activated caspase-3 cleaves DFF45 and activates DFF40, a DNase that is thought to cleave DNA into nucleosomal fragments. Interestingly, Bid overexpression led to Bid processing in the absence of additional exogenous apoptotic protease activity.
stimuli, suggesting enhanced caspase activation following viral infection or Bid overexpression. Because full-length Bid can translocate to the mitochondrial membrane (12), resulting in cytochrome c release, caspase activation, and processing of tBid, increased caspase activation is expected in Ad-Bid-infected cells irrespective of tBid processing. To determine whether Ad-Bid infection resulted in caspase activation, caspase-3 and -9 activities were measured in H460 cells after infection. In two independent experiments, transduction with Ad-LacZ induced an approximately 2-fold induction of caspase-3 and -9, whereas Ad-Bid consistently induced a 4–5-fold induction of these caspases (Fig. 2A). Using a paired t test, the levels of caspase-3 and -9 were significantly different from mock controls (t = 0.01 and 0.009, respectively). For caspase-3, both Ad-LacZ and Ad-p53 significantly enhanced caspase activity compared with mock controls (t = 0.04 and 0.03, respectively), whereas only Ad-Bid significantly enhanced caspase-9 activity compared with mock controls (using a paired t test, t = 0.2 and 0.1 for Ad-LacZ and Ad-p53, respectively). As a positive control, Jurkat cells treated with soluble Fas ligand are shown in Fig. 2A (5–7-fold increase in caspase-3 and -9, respectively). Caspase-8 activation was not detectable in Ad-Bid-infected cells (data not shown), implying that under these experimental conditions there was little activation of the death receptor pathway. These data suggest that Ad-Bid may activate effector caspases (such as caspase-3 and -9) through a mitochondrial death pathway to enhance Bid processing. In support of this premise, treatment of Ad-Bid-infected cells with the general caspase inhibitor zVAD-fmk inhibited cleavage of Bid to tBid (Fig. 2B), whereas the caspase-8 inhibitor IETD-fmk was unable to block this cleavage (data not shown). In addition, zVAD-fmk blocked DNA cleavage in Ad-Bid-infected cells (Fig. 2C), suggesting that tBid processing by effector caspases played a crucial role in Ad-Bid-induced apoptosis.

Mitochondria-associated Changes in NSCLC Induced by Ad-Bid—Activation of effector caspase-3 and -9 suggested that Ad-Bid was inducing mitochondrial membrane changes and cytochrome c release like that observed with Bax and Bak overexpression (15, 16). Because BH-3 domain-only proteins such as Bid require at least one Bax-like partner to induce apoptosis (8), we examined Bak and Bax expression in the three lung cancer cell lines by Western blotting. Bax was expressed equivalently in all three cell lines (Fig. 3A), whereas Bak expression was considerably more variable with A549 cells expressing low levels as reported (16).

To determine whether Ad-Bid infection corresponded with changes in mitochondrial permeability, cell lines were treated with the fluorescent dye DiOC₆ to target mitochondrial events. As shown in Fig. 3B, Ad-Bid caused a significant reduction in DiOC₆ fluorescence, indicating mitochondrial depolarization.
The changes elicited by Ad-Bid treatment were more striking than those observed with anti-Fas. These events were accompanied by a sharp increase in cytosolic cytochrome c detectable by Western blotting (Fig. 3C) and seen only in Ad-Bid-treated cells and not in either mock-infected or tetracycline-treated Ad-Bid-infected cells. The mitochondria-associated protein Cox4 is shown as a control. Experiments were repeated three times with similar findings.

DNA Fragmentation and Death in Ad-Bid-infected NSCLC—To document that mitochondrial membrane changes were accompanied by the induction of cell death, lung cancer cell lines were examined for sub-G1 DNA content using propidium iodide staining and flow cytometry 48 h after Ad-Bid infection. Infection with the Ad-LacZ control resulted in little, if any, DNA fragmentation (range 0.8–7.8%), whereas Ad-Bid infection resulted in a marked increase in sub-G1 DNA content (range 11.0–80.0%) in all three cell lines (Fig. 4A). For the sake of comparison, DNA fragmentation also is shown for Ad-p53, a therapy currently in clinical trials for lung cancer and head and neck cancer (17–19). Ad-Bid was consistently more effective than Ad-p53 in the induction of DNA fragmentation and cell death (Fig. 4A) at comparable multiplicities of infection. Such effects were most striking in H460 and A549 cells, where Ad-p53 resulted in ~20% loss of cell viability 48 h following infection with 5 m.o.i. of virus, whereas Ad-Bid at the same viral dose and time resulted in ~90% cell death. In H358 cells, which are relatively resistant to Ad-p53-induced apoptosis (~10% loss of cell viability), Ad-Bid induced >50% cell death. Similar findings were observed in three independent experiments.

Enhanced Apoptosis in NSCLC Infected with Ad-Bid Treated with Cisplatin or Anti-Fas—The goal of preclinical studies is to maximize tumor cell killing, particularly in more resistant tumors such as the p53-null tumor H358. Because Bid can be processed by caspase-8 activation, we reasoned that enhanced killing might be observed with Ad-Bid in combination with activators of caspase-8. To this end, we examined the combined effects of Ad-Bid and either cisplatin or anti-Fas on tumor cell killing. Both anti-Fas and cisplatin treatment activated caspase-8 in H460 and H358 cells. In H460 cells, anti-Fas induced an approximately 5-fold increase in caspase-8 activity, whereas cisplatin induced a ~4–5-fold increase (Fig. 5A). In H358 cells, anti-Fas and cisplatin treatment resulted in a more modest activation of caspase-8 with a ~1.5–2-fold activation by anti-Fas and a ~2–3-fold activation by cisplatin. As a positive control, caspase-8 activation is shown for both cell lines following treatment with soluble Fas ligand (Fig. 5A). In H358 cells, anti-Fas and cisplatin treatment resulted in a more modest activation of caspase-8 with a 1.5–2-fold activation by anti-Fas and a 2–3-fold activation by cisplatin. As a positive control, caspase-8 activation is shown for both cell lines following treatment with soluble Fas ligand (Fig. 5A). These experiments were repeated twice with similar findings. H358 and H460 cell lines express low levels of Fas and are moderately sensitive to anti-Fas antibody, in both cases, anti-Fas treatment re-
Ad-Bid Induces Apoptosis in NSCLC

Recent clinical trials have demonstrated the feasibility of using adenovirally mediated gene transfer of proapoptotic genes such as p53 either alone or in combination with radiation or chemotherapy to treat advanced lung cancer and recurrent head and neck tumors that are normally resistant to conventional therapies (17–19). Although a number of patients have shown evidence of clinical responses using this approach, some individuals fail to respond, respond poorly, or show disease progression, stressing a need for additional proapoptotic candidate genes. In this report, we have used a Tet-Off™ adenoviral expression system containing human full-length Bid to achieve high Bid levels and potent apoptosis in NSCLC cell lines. To our knowledge, this is the first report using adenovirally expressed Bid to induce apoptosis in malignant cells. As a therapeutic gene for NSCLC, Bid is particularly attractive as apoptosis occurs independently of the status of p53 (p53 mutations have been observed in ~80% of NSCLC (22)). Furthermore, apoptosis can occur in cells expressing high levels of antiapoptotic Bcl-2 family members (16) (~48% of NSCLC have been shown to overexpress Bcl-2 (23)).

Our studies suggest that Bid overexpression in NSCLC cell lines results in potent apoptosis as measured by changes in mitochondrial transmembrane potential, cytochrome c release, DNA fragmentation, and loss of cell viability (Fig. 3, B and C, and Fig. 4). Cell death induced by Ad-Bid is p53-independent in that Bid effectively kills both p53 wild-type (A549, H460) and p53-null (H358) cells, suggesting that Bid, like other Bcl-2 family members, functions downstream of p53. Ad-Bid can induce apoptosis in NSCLC cells expressing high levels of antiapoptotic Bcl-2 family members (16) (~48% of NSCLC have been shown to overexpress Bcl-2 (23)).

DISCUSSION

Recent clinical trials have demonstrated the feasibility of using adenovirally mediated gene transfer of proapoptotic genes such as p53 either alone or in combination with radiation or chemotherapy to treat advanced lung cancer and recurrent head and neck tumors that are normally resistant to conventional therapies (17–19). Although a number of patients have shown evidence of clinical responses using this approach, some individuals fail to respond, respond poorly, or show disease progression, stressing a need for additional proapoptotic candidate genes. In this report, we have used a Tet-Off™ adenoviral expression system containing human full-length Bid to achieve high Bid levels and potent apoptosis in NSCLC cell lines. To our knowledge, this is the first report using adenovirally expressed Bid to induce apoptosis in malignant cells. As a therapeutic gene for NSCLC, Bid is particularly attractive as apoptosis occurs independently of the status of p53 (p53 mutations have been observed in ~80% of NSCLC (22)). Furthermore, apoptosis can occur in cells expressing high levels of antiapoptotic Bcl-2 family members (16) (~48% of NSCLC have been shown to overexpress Bcl-2 (23)).

Our studies suggest that Bid overexpression in NSCLC cell lines results in potent apoptosis as measured by changes in mitochondrial transmembrane potential, cytochrome c release, DNA fragmentation, and loss of cell viability (Fig. 3, B and C, and Fig. 4). Cell death induced by Ad-Bid is p53-independent in that Bid effectively kills both p53 wild-type (A549, H460) and p53-null (H358) cells, suggesting that Bid, like other Bcl-2 family members, functions downstream of p53. Ad-Bid can induce apoptosis in NSCLC cells expressing high levels of antiapoptotic Bcl-2 family members (16, 23), suggesting that Bid can overcome Bcl-2 inhibition of the Bak and Bax conformational changes required for the induction of cell death (24–28). Because Bid can induce apoptosis in partnership with either Bak or Bax, Ad-Bid also may be effective in tumors containing mutations in one of these proteins (29, 30).

NSCLC cell lines infected with Ad-Bid express large quantities of Bid protein (Fig. 1A). When Bid was overexpressed, both full-length Bid (24 kDa) and tBid (15 kDa) were detectable by Western blotting (Fig. 1A). Detection of tBid was dependent on the viral dose used for infection (Fig. 1B) with tBid observed at viral doses of 5 m.o.i. or greater. Interestingly, Ad-Bid infection consistently induced both caspase-9- and caspase-3-like activity in NSCLC cells at levels 4–5-fold above that observed with mock-infected cells (Fig. 2A). In NSCLC cell lines infected with

sulted in ~90% of the cells (consistent with Fig. 4), and the addition of anti-Fas further diminished viable cell counts to <5% of the starting population. In the case of the more resistant cancer cell line H358, Bid overexpression resulted in the death of ~50% of the cells (consistent with Fig. 4), and the addition of anti-Fas diminished viabilities to <5% of the starting population. Likewise, when Bid was overexpressed, both cell lines showed a marked sensitivity to cisplatin. Ad-LacZ transduced H460 cells showed ~60% cell death after treatment with cisplatin, whereas the Ad-Bid and cisplatin combination induced ~95% cell death (Fig. 5C). In the H358 lung cancer cells, Ad-LacZ treatment in combination with cisplatin induced ~35% cell death, whereas the Ad-Bid and cisplatin combination increased killing to ~90%. These experiments were repeated four times with similar findings. As expected based on the loss of cellular viability, Ad-Bid in combination with anti-Fas or cisplatin also increased DFF45 cleavage (Fig. 6A) and tBid processing (Fig. 6B) in both cell lines (n = 2). Taken together, these data suggest that agents capable of activating caspase processing of Bid may enhance Ad-Bid-induced tumor cell killing in an additive or superadditive manner.

![Fig. 5. NSCLC lines show increased caspase-8-like activity and cell death following Ad-Bid in combination with cisplatin and anti-Fas treatment.](http://www.jbc.org/)

A.

B.

C.

sulted in 40% cell killing. When Bid was overexpressed, however, both lung cancer cell lines showed a marked enhancement of Fas sensitivity. In the case of H460, Ad-Bid treatment re-
Ad-Bid Induces Apoptosis in NSCLC

Because Bid is recognized as an intracellular link connecting death receptors and the mitochondrial apoptotic pathway through caspase-8 activation (4, 5), Ad-Bid-induced apoptosis should be enhanced by agents that increase caspase-8 and anti-Fas. As shown in Fig. 5A, both agents activated caspase-8 activity in NSCLC cell lines and potentially enhanced cell killing by Bid (Fig. 5B and C). As expected with increased caspase-8 activity, tBid and DFF45 cleavage also were elevated in the combination Ad-Bid treatments (Fig. 6). Of particular note is the restoration of chemosensitivity to cisplatin in p53-null H358 after Ad-Bid treatment. These cells normally are resistant to cisplatin killing, showing about 35% cell death when treated with the control virus Ad-LacZ and cisplatin (2.5 μg/ml). Ad-Bid alone induced ~50% cell death, whereas the combination of Ad-Bid and cisplatin resulted in >90% loss of cell viability. These studies suggest that Ad-Bid can induce chemosensitization in the absence of functional p53 and that Ad-Bid in combination with cisplatin or Fas receptor engagement might offer a strategy to maximize the apoptotic signal in malignant cells, including those lacking p53 function.

In summary, we have shown that adenovirally mediated overexpression of Bid leads to the rapid and potent activation of apoptosis in malignant NSCLC cell lines in a p53-independent manner and in the presence of high levels of antiapoptotic Bcl-2 family members. Caspase-dependent processing of tBid occurs in Ad-Bid-infected cells and can be enhanced further through caspase-8 (cisplatin, Fas engagement), leading to additive or superadditive cell killing. These results suggest that gene transfer with Bid alone or Bid in combination with either chemotherapy or death receptor engagement may be a useful strategy for the treatment of malignant disease. Because Bid killing is non-selective (i.e. not specific to malignant cells), the development of tissue- and tumor-specific promoter systems will be required to ensure specificity and limit death in normal tissues. Additional studies currently are underway to develop Ad-Bid therapeutic vectors that can be used for preclinical evaluation.

Acknowledgments—We thank colleagues at the University of Texas M. D. Anderson Cancer Center for expert technical assistance in the production of high titer adenoviral stocks (Trupti Mehta and Nancy Yen). We also thank Dr. Jack Roth and laboratory members for critical and stimulating discussions related to these studies.

REFERENCES

1. Green, D. R., and Evan, G. I. (2002) Cancer Cell 1, 19–30
2. Johnstone, R. W., Rüedi, A. A., and Lowe, S. W. (2002) Cell 109, 153–164
3. Green, D. R., and Reed, J. C. (1998) Science 281, 1309–1313
4. Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998) Cell 94, 481–490
5. Li, H., Zhu, H., Xu, C. J., and Yuan, J. (1998) Cell 94, 491–501
6. Adams, J. M., and Cory, S. (2001) Trends Biochem. Sci. 26, 61–66
7. Desagher, S., and Martinou, J. C. (2000) Trends Cell Biol. 10, 369–377
8. Zong, W.-X., Lindsten, T., Ross, A. J., MacGregor, G., and Thompson, C. B. (2001) Genes Dev. 15, 1481–1486
9. Wei, M. C., Zong, W.-X., Cheng, E. H.-Y., Lindsten, T., Panoutsakopoulou, V., Ross, A. J., Roth, K. A., MacGregor, G. R., Thompson, C. B., and Korsmeyer, S. J. (2001) Science 292, 727–730
10. Wang, K., Yin, X. M., Chao, D. T., Milliman, C. L., and Korsmeyer, S. J. (1994) Cancer Res. 54, 2287–2291
11. Tafani, M., Karpinich, N. O., Hurster, K. A., Pastorino, J. G., Schneider, T., Russo, M. A., and Farber, J. L. (2002) J. Biol. Chem. 277, 10973–10983
12. Fujisawa, T., Grimm, E. A., Mukhopadhyay, T., Owen-Schaub, L. B., and Roth, J. A. (1994) Cancer Res. 54, 2287–2291
13. Zamzami, N., Marchetti, P., Castedo, M., Zanin, C., Vayssiere, J. L., Petit, P. X., and Kroemer, G. (1995) J. Exp. Med. 181, 1661–1672
14. Kagawa, S., Gu, J., Swisher, S. G., Li, J., Roth, J. A., Lai, D., Stephens, L. C., and Fang, B. (2000) Cancer Res. 60, 1157–1161
15. Pataer, A., Fang, B., Yu, R., Kagawa, S., Hunt, K. K., McDowell, T. J., Roth, J. A., and Swisher, S. G. (2000) Cancer Res. 60, 788–792
16. Clayman, G. L., El-Naggar, A. K., Lippman, S. M., Henderson, V. C., Frederick, M., Merritt, J. A., Zumstein, L. A., Timmons, T. M., Liu, T.-J., Ginsberg, L., Roth, J. A., Hong, W. K., Bruso, P., and Goeppert, H. (1998) J. Clin. Oncol. 16, 2221–2232
18. Swisher, S. G., Roth, J. A., Nemunaitis, J., Lawrence, D. D., Kemp, B. L., Carrasco, C. H., Connors, D. G., El-Naggar, A. K., Fossella, F., Glisson, B. S., Hong, W. K., Khuri, F. R., Kurie, J. M., Lee, J. J., Lee, J. S., Mack, M., Merritt, J. A., Nguyen, D. M., Neshitt, J. C., Perez-Soler, R., Pisters, K. M. W., Putnam, J. B., Jr., Richi, W. R., Savin, M., Schrump, D. S., Shin, D. M., Shulkin, A., Walsh, G. L., Wu, J., Weil, D., and Waugh, M. K. A. (1999) *J. Natl. Cancer Inst.*, 91, 763–771
19. Swisher, S. G., Roth, J. A., Komaki, R., Gu, J., Lee, J. J., Hicks, M., Ro, J. Y., Hong, W. K., Merritt, J. A., Ahrar, K., Atkinson, N. E., Correa, A. M., Delsert, D., Dreiling, L., El-Naggar, A. K., Fossella, F., Fracisco, R., Glisson, B., Grammar, S., Herb, R., Huaringa, A., Kemp, B., Khuri, F. R., Karine, J. M., Liao, Z., McDonnell, T. J., Morice, R., Morello, F., Putnam, J. B., Jr., Sarabia, A. J., Shelton, T., Stevens, C., Shin, D. M., Smythe, W. R., Vaias, A. A., Walsh, G. L., and Yin, M. (2003) *Clin. Cancer Res.* 9, 931–939
20. Owen-Schaub, L. B., Zhang, W., Cusack, J. C., Angelo, L. S., Santee, S. M., Fujiwara, T., Roth, J. A., Deisseroth, A. B., Zhang, W.-W., Kruzel, E., and Radinsky, R. (1995) *Mol. Cell. Biol.* 15, 3082–3092
21. Owen-Schaub, L., Chan, H., Cusack, J. C., Roth, J. A., and Hill, L. L. (2000) *Int. J. Oncol.* 17, 5–12
22. Fujita, T., Kiyama, M., Tomizawa, Y., Kohno, T., and Yokota, J. (1999) *Int. J. Oncol.* 15, 927–934
23. Landau, D., Pinkins, J., Surzykowski, L., Chyczewski, L., and Nikiforos, A. (2001) *Eur. Respir. J.* 17, 660–666
24. Nechustan, A., Smith, C. L., Haus, Y. T., and Youle, R. J. (1999) *EMBO J.* 18, 2330–2341
25. Griffins, G. J., Dubez, L., Morgan, C. P., Jones, N. A., Whitehouse, J., Corfe, B. M., Dive, C., and Hickman, J. C. (1999) *Cell Biol.* 144, 495–514
26. Wei, M. C., Lindsten, T., Moeha, V. K., Weiler, S. S., Gross, A. E., Ashiya, M., Thompson, C. B., and Korsmeyer, S. J. (2000) *Genes Dev.* 14, 2060–2071
27. Antonsen, B., Montessuit, S., Sanchez, B., and Martinou, J. C. (2001) *J. Biol. Chem.* 276, 11615–11623
28. Mikhailov, V., Mikhailova, M., Kulkov, D. J., Dong, Z., Venkatachalam, M. A., and Saikumar, P. (2001) *J. Biol. Chem.* 276, 18361–18374
29. Brimmell, M., Mendola, R., Mangion, J., and Packham, G. (1998) *Oncogene* 16, 1803–1812
30. Kondo, S., Shinozuma, Y., Miyazaki, Y., Kiyohara, T., Tsutsui, S., Kitamura, S., Nagasawa, Y., Nakaahara, M., Kanayama, S., and Matsuzawa, Y. (2000) *Cancer Res.* 60, 4328–4330
Adenoviral Bid Overexpression Induces Caspase-dependent Cleavage of Truncated Bid and p53-independent Apoptosis in Human Non-small Cell Lung Cancers
Takuya Fukazawa, Barbara Walter and Laurie B. Owen-Schaub

J. Biol. Chem. 2003, 278:25428-25434.
doi: 10.1074/jbc.M302058200 originally published online April 10, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302058200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 20 of which can be accessed free at
http://www.jbc.org/content/278/28/25428.full.html#ref-list-1