Nicotine Induces Multi-site Phosphorylation of Bad in Association with Suppression of Apoptosis*

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Nicotine is an important component in cigarette smoke that can activate the growth-promoting pathways to facilitate the development of lung cancer. However, the intracellular mechanism(s) by which nicotine promotes survival of lung cancer cells remains enigmatic. Bad is a proapoptotic BH3-only member of the Bcl2 family and is expressed in both small cell lung cancer and non-small cell lung cancer cells. Here we report that nicotine potently induces Bad phosphorylation at Ser112, Ser136, and Ser155 in a mechanism involving activation of MAPKs ERK1/2, PI3K/AKT, and PKA in human lung cancer cells. Nicotine-induced multi-site phosphorylation of Bad results in sequestering Bad from mitochondria and subsequently interacting with 14-3-3 in the cytosol. Treatment of cells with PKC inhibitor (staurosporine), MEK-specific inhibitor (PD98059), PI3K kinase inhibitor (LY294002), or PKA inhibitor (H89) blocks the nicotine-induced Bad phosphorylation that is associated with enhanced apoptotic cell death. The fact that β-adrenergic receptor inhibitor (propranolol) blocks nicotine-induced activation of ERK1/2, AKT, PKA, Bad phosphorylation, and cell survival suggests that nicotine-induced Bad phosphorylation may occur through the upstream β-adrenergic receptors. The fact that specific knockdown of Bad expression by RNA interference using short interfering RNA enhances cell survival and that nicotine has no additional survival effect on these cells suggests that Bad may act as a required target of nicotine. Thus, nicotine-induced survival may occur in a mechanism through multi-site phosphorylation of Bad, which may lead to development of human lung cancer and/or chemoresistance.

Lung cancer is one of the leading causes of cancer death, with a 5-year relative survival rate of 15% in both men and women worldwide (1–2). Cigarette smoking is by far the most important risk factor in the development of lung cancer (3). About 90% of male lung cancer deaths and 75–80% female lung cancer deaths in the United States are caused by smoking (1, 4). Approximately 25% of the U. S. adult population continue to smoke because of addiction to nicotine, one important component of cigarette smoking (5–6).

Nicotine can activate the growth-promoting pathways to facilitate the development of lung cancer and potentially reduce the efficacy of chemotherapeutic agents (7). Although classic nicotine signaling occurs through the nicotinic acetylcholine receptor (nAChR) (8–9), growing evidence suggests that the adrenergic receptor also plays an important role in nicotine signal transduction pathways, especially in pulmonary adenocarcinoma cells (10–11).

Bel-2 family members are key regulators of apoptosis, and their deregulation could be oncogenic (12–13). Recent observations suggest that responsiveness to therapy and prognosis of lung cancer may be associated with the Bel-2 family proteins (14).

Bel-2 family has at least 20 members in mammals, all of which share at least one Bel-2 homology (BH) domain (13). Among these domains, the BH3 domain is responsible for interaction with other Bel2 family proteins and promotes cells undergoing apoptosis (15). Bel2 family members control cell survival or cell death by regulating the mitochondrial pathway (2–3). Bel-2 proteins such as Bcl-2 and Bcl-xL can act as a protective action of Bcl-xL (16). Bel-2 proteins (i.e. Bel, Bid, Bik, Bim, Nix, and Noxa, etc.) may couple death signals to mitochondria and promote apoptosis by quelling the mitochondrial membrane permeabilization (16). The BH3-only proapoptotic proteins (i.e. Bel, Bad, Bik, Bid, Bim, Nix, and Noxa, etc.) can couple death signals to mitochondria and promote apoptosis by quelling the mitochondrial membrane permeabilization (16). The BH3-only proapoptotic proteins (i.e. Bel, Bad, Bik, Bid, Bim, Nix, and Noxa, etc.) may couple death signals to mitochondria and promote apoptosis by quelling the mitochondrial membrane permeabilization (16).

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* The abbreviations used are: nAChR, nicotinic acetylcholine receptor; BH, Bel-2 homology; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PKC, protein kinase c; PKA, cAMP-dependent protein kinase A; SCLC, small cell lung carcinoma; siRNA, short interfering RNA; PI3K, phosphatidylinositol 3'-OH kinase; α-BTX, α-bungarotoxin.

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survival and chemoresistance may occur, at least in part, through phosphorylation of Bad.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bad, ERK1, ERK2, AKT1/2, phospho-specific ERK, phospho-specific Bad (Ser136/Ser135) 14-3-3-8 (K-19) antibodies, as well as PKA inhibitor H89 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-specific Bad (Ser155) and phospho-specific AKT antibodies were obtained from Cell Signaling Technology (Beverly, MA). Nicotine, propranolol, etoposide (VP16) and cisplatin were purchased from Sigma. Puromycin, FD88065, staurosporine, LY294002, and phospho-specific pan-Ing familial members were purchased from Calbiochem (San Diego, CA). A549 and NCI-H23 cell lines were obtained from American Type Culture Collection (Manassas, VA). SigmaTACT CAM-dependent protein kinase (PKA) assay kit was purchased from Promega (Madison, WI). All reagents used were obtained from commercial sources unless otherwise stated.

**Cell Lines and Cell Culture**—A549 cells were maintained in F-12K medium with 10% fetal bovine serum and 4 mM L-glutamine. NCI-H69, NCI-H82, NCI-H157, NCI-H23, NCI-H358, and NCI-H460 cells were maintained in RPMI 1640 medium with 10% fetal bovine serum.

**Protein Isolation**—Cells were washed with phosphate-buffered saline and resuspended in ice-cold EBC buffer (0.5% Nonidet P-40, 50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, and 1 mM β-mercaptoethanol) with protease inhibitor mixture set I (Calbiochem) and lysed by sonication. The lysates were cleared of insoluble material by centrifugation at 14,000 × g for 10 min at 4 °C.

**Metabolic Labeling, Immunoprecipitation, and Western Blot Analysis**—Cells were washed with phosphate-free RPMI medium 1640 and metabolically labeled with [32P]orthophosphoric acid for 90 min. After treatment, cells were washed with ice-cold phosphate-buffered saline and lysed in detergent buffer. Bad was immunoprecipitated using Bad antibody, as described previously (29–30). The samples were subjected to SDS–12% PAGE, transferred to a nitrocellulose membrane, and exposed to Kodak X-omat film at 80 °C. Bad phosphorylation was determined by autoradiography. The autoradiograph was then probed by Western blot analysis with a Bad antibody and developed by using an ECL Kit (Amersham Biosciences), as described previously (30).

**Assay of PKA Activity in Vitro**—PKA activity was measured using a PKA assay kit according to the manufacturer’s instructions (31). A549 cells were treated with nicotine in the presence or absence of inhibitor(s). Cells were then lysed using PKA extraction buffer. Enzyme sample (5 μl) was added and incubated at 30 °C for 5 min. Reaction buffer (10 μl) for termination was placed onto a pre-numbered membrane filter. After washing and rinsing, the membrane was dried, placed into a scintillation vial, and counted by scintillation counting.

**Cell Viability Assay**—The apoptotic and viable cells were detected using an ApoAlert Annexin-V kit (Clontech) according to the manufacture’s instructions. The percentages of Annexin-Vlow cells (percentage of viable cells) or Annexin-Vhigh cells (percentage of apoptotic cells) were determined by using the data obtained by fluorescence-activated cell sorter analysis as described (32). Cell viability was also confirmed by using the trypan blue dye exclusion method (29).

**Subcellular Fractionation**—Cells (2 × 10^6) were washed with cold 1× phosphate-buffered saline and resuspended in isotonic mitochondrial buffer (210 mM mannitol, 70 mM sucrose, 1 mM EGTA, 10 mM Hepes, pH 7.5) containing protease inhibitor mixture set I, homogenized with a polytron homogenizer operating for four bursts of 10 s each for 3 min to pellet the nuclei and unbroken cells. The supernatant was centrifuged at 13,000 × g for 10 min to pellet the mitochondria as described (33). The second supernatant was further centrifuged at 150,000 × g for 1 hr to pellet light membranes. The resulting supernatant is the cytosolic fraction. Mitochondria were washed with mitochondrial buffer twice, resuspended in 1% Nonidet P-40 lysis buffer, rocked for 60 min, and then centrifuged at 14,000 rpm for 10 min at 4 °C. The resulting supernatant containing mitochondrial proteins was collected. Protein (100 μg) from each fraction was subjected to SDS-PAGE. Bad or phosphorylated Bad was analyzed by Western blot using Bad or phospho-specific Bad antibody, respectively. The purity of fractions was confirmed by assessing localization of mitochondria-specific protein, prohibitin (34).

**Co-Immunoprecipitation**—A549 cells expressing high levels of endogenous Bad were transfected with BAD siRNA according to the siPORT lipid reagent instructions. The percentages of Annexin-Vlow cells (percentage of viable cells) or Annexin-Vhigh cells (percentage of apoptotic cells) were determined by using the data obtained by fluorescence-activated cell sorter, as described previously (32). Data represent the mean ± S.D. of three determinations.

**RESULTS**

**Nicotine Induces Bad Phosphorylation in Association with Suppression of apoptosis in human lung cancer cells.** A, expression levels of endogenous Bad or Bcl2 in various human lung cancer cell lines were analyzed by Western blotting using Bad or Bcl2 antibody, respectively. B, A549 cells expressing high levels of endogenous Bad were metabolically labeled with [32P]orthophosphoric acid and treated with nicotine (1 μM) for various times as indicated. Bad was immunoprecipitated using Bad antibody. Phosphorylation of Bad was determined by autoradiography (top). Western blot analysis was performed to confirm and quantify Bad protein (bottom). C, A549 cells were treated with cisplatin (40 μM) or VP-16 (50 μM) in the absence or presence of nicotine for 24 h. Samples were harvested and analyzed for Annexin-V and phosphatidylinositol binding by flow cytometry. Cell viability was determined by fluorescence-activated cell sorter, as described previously (32). Data represent the mean ± S.D. of three independent experiments.

**Nicotine Induces Bad Phosphorylation in Association with Increased Survival of Human Lung Cancer Cells**—The apoptotic process can be divided into three interdependent phases: induction, decision, and execution. The decision phase is mainly regulated by the Bcl-2 family of apoptotic regulators (35). We have recently demonstrated that nicotine induces Bcl-2 phosphorylation at Ser^155^ and potently promotes survival of human small cell lung cancer (SCLC) H69 cells (23). Bad is one of the BH3-only Bcl2 family members, and phosphorylation negatively regulates its proapoptotic activity (22). Interestingly, Bad is more widely expressed in human lung cancer cells, including SCLC and non-small cell lung cancer cells, than Bcl2 (Fig. 1A). This finding suggests that Bad may play a more important role in nicotine-induced survival and chemoresistance of human lung cancer cells, especially in those cells that express low or undetectable levels of endogenous Bcl2. To test whether nicotine can induce Bad phosphorylation and promote cell survival, A549 cells (i.e. human pulmonary adenocarcinoma cell line) expressing high levels of endogenous Bad but no detectable levels of Bcl-2 were tested (Fig. 1A). A549 cells were metabolically labeled with [32P]orthophosphoric acid and treated with nicotine (1 μM) for various times as indicated. Results indicate that nicotine potently stimulates Bad phos-
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phorylation, which could abolish the proapoptotic function of Bad (Fig. 1B; Ref. 20). Similar experiments were also performed by using other lung cancer cell lines (i.e. H157 and H358 cells), and similar results were obtained (data not shown). Because nicotine prolongs cell survival after treatment with cisplatin or VP-16 by 30–40% (Fig. 1C), this increased survival may result, at least in part, from phosphorylation of Bad.

Nicotine Induces Bad Phosphorylation at Ser\(^{112}\), Ser\(^{136}\), and Ser\(^{155}\)—Bad phosphorylation at Ser\(^{112}\), Ser\(^{136}\), and Ser\(^{155}\) abrogates the proapoptotic function of Bad (20, 36). Results from metabolic labeling experiments show that nicotine can induce Bad phosphorylation (Fig. 1B), but which are the phosphorylation sites remains unclear. To identify nicotine-induced Bad phosphorylation sites, A549 cells were treated with nicotine (1 \(\mu\)M) for various times (A) or treated with various concentrations of nicotine for 30 min (B), as indicated. Phosphorylation of Bad was analyzed by Western blotting using phospho-specific Ser\(^{112}\), Ser\(^{136}\), and Ser\(^{155}\) antibody, respectively. Bad antibody was used to confirm and quantify Bad protein.

induced multi-site Bad phosphorylation may occur through activation of multiple physiological Bad kinases including MAPKs ERK1/2, AKT, and PKA.

Staurosporine, PD98059, LY294002, and H89 Inhibit Nicotine-induced Bad Phosphorylation and Promote Apoptosis—Because phosphorylation inactivates the proapoptotic function of Bad (20, 36), it is possible that inhibition of Bad phosphorylation may restore its proapoptotic effect. To test this possibility, specific inhibitors for Bad kinases were used. A549 cells were incubated with nicotine (1 \(\mu\)M) in the presence or absence of various concentrations of staurosporine (a PKC inhibitor; Ref. 37), PD98059 (an MEK-specific inhibitor; Ref. 38), LY294002 (a PI3 K-specific inhibitor; Ref. 39), or H89 (a PKA-specific inhibitor; Ref. 40). Results indicate that staurosporine or PD98059 potently blocks nicotine-induced MAPKs ERK1/2 activation as well as Ser\(^{112}\) site phosphorylation of Bad (Fig. 4, A and B). This suggests that nicotine may trigger a PKC/ERK1/2 protein kinase cascade to phosphorylate and inactivate Bad. In addition, LY294002 or H89 also inhibits nicotine-induced Bad phosphorylation at Ser\(^{136}\) or Ser\(^{155}\), respectively (Fig. 4, C and D). These findings provide the pharmacological evidence that AKT and PKA are also involved in nicotine/Bad signaling. Importantly, staurosporine, PD98059, LY294002, and H89 potently block nicotine-induced cell survival after treatment with chemotherapeutic agents (i.e. cisplatin, VP16; Fig. 4E). These results indicate that inhibition of nicotine-induced Bad phosphorylation by blocking these three upstream pathways (i.e. PKC/ERKs, PI3 K/AKT, and PKA) restores the proapoptotic activity of Bad that triggers apoptotic cell death.

The \(\beta\)-Adrenergic Receptor-specific Inhibitor Propranolol Potently Inhibits Nicotine-induced Bad Phosphorylation and Enhances Apoptosis—It has been reported that nAChRs, espe-
cially α7nAChR and β-adrenergic receptor, play important roles in nicotine signaling in lung cancer cells (6, 8, 10, 41). α-Bungarotoxin (α-BTX), a non-competent potent α7nAChR-specific inhibitor (42) and propranolol, a β-adrenergic receptor inhibitor (43), were selected to test which type of receptor may be involved in nicotine/Bad signaling. A549 cells were treated with nicotine in the presence or absence of various concentrations of propranolol or α-BTX. Interestingly, propranolol but not α-BTX can completely block nicotine-induced MAPKs ERK1/2, AKT, and PKA activation and Bad phosphorylation at Ser112, Ser136, and Ser155 sites (Fig. 5 and data not shown). Functionally, propranolol blocks nicotine-induced cell survival

Fig. 4. Staurosporine, PD98059, LY294002, or PKA inhibitor H89 inhibits nicotine-induced activation of MAPKs ERK1/2, AKT, or PKA in association with decreased Bad phosphorylation and increased apoptosis. A–D, A549 cells were treated with nicotine (1 μM) in the absence or presence of various concentrations of Stauro, PD98059, LY294002, or H89 for 30 min. Phosphorylation of ERK1/2, AKT, or Bad was analyzed by Western blotting using phospho-specific ERK, AKT, or Bad antibody, respectively, as described in Figs. 2 and 3. E and F, A549 cells were treated with cisplatin (40 μM) or VP16 (50 μM) in the absence or presence of nicotine (1 μM) and/or Stauro (0.1 μM), PD98059 (20 μM), LY294002 (20 μM), or H89 for 24 h. Cell viability was analyzed as described in Fig. 1. Data represent the mean ± S.D. of three determinations.
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and promotes apoptosis after treatment with cisplatin or VP-16. These results implicate nicotine-induced Bad phosphorylation in a mechanism involving the upstream β-adrenergic receptor in pulmonary adenocarcinoma cells.

Nicotine Induces Bad Translocation from Mitochondria into Cytosol, Which Facilitates Association with 14-3-3—Our results indicate that nicotine induces multi-site phosphorylation of Bad in association with increased cell survival. Phosphorylation has been reported to promote Bad translocation from mitochondria into cytosol and interaction with the scaffold protein 14-3-3 (18, 21). To assess whether nicotine affects Bad translocation, A549 cells were treated with nicotine for 30 min. Subcellular fractionation experiments were performed to isolate mitochondria and cytosol, as described under “Experimental Procedures.” Results reveal that nicotine potently stimulates translocation of Bad from mitochondria into cytosol (Fig. 6A). Phosphorylated Bad is observed only in cytosol, and its accumulation is increased in a dose-dependent manner (Fig. 6A). Immunoprecipitation experiments show that nicotine enhances the association between Bad and 14-3-3. These results reveal that nicotine-induced multi-site Bad phosphorylation results in sequestering Bad from mitochondria and functionally blocking its proapoptotic function.

Bad May Be a Required Target for Nicotine-induced Survival of Human Lung Cancer Cells—Bad is ubiquitously expressed in human lung cancer cell lines, whereas Bcl2 is not (Fig. 1A), suggesting that Bad may be a more important potential death target for treatment of patients with lung cancer. To test whether Bad is a necessary target for nicotine-induced survival and chemoresistance of human lung cancer cells, an RNA interference approach was employed. It has recently been demonstrated that 21 base pair double-strand RNA (siRNA) is a potent mediator of the RNA interference effect in mammalian cells (44). We used this strategy to target Bad in A549 cells by transfecting cells with Bad siRNA, as described under “Experimental Procedures.” Results show that the Bad siRNA can potently and specifically reduce Bad expression by more than 90%, whereas the control siRNA has no effect (Fig. 7A). Depletion of Bad expression was found to prolong cell survival after treatment with chemotherapeutic agents (i.e. cisplatin or VP-16) in the absence or presence of nicotine (Fig. 7B). Nicotine has no additional survival effect in cells expressing Bad siRNA (Fig. 7), indicating that Bad may be a required target for nicotine/survival signaling in human lung cancer cells.

DISCUSSION

Bcl-2 and related family proteins are major regulators of apoptosis that are critical for development and tumorigenesis in autoimmune and degenerative diseases (13). Bad belongs to the BH3-only Bcl-2 subfamily and shares the conserved BH3 domain (i.e. death domain) with other Bcl2 family members (13, 45). All members of this BH3-only subfamily are proapoptotic and have been shown to interact with one or more of the death suppressor family members, such as Bcl-XL (46). Phosphorylation, a post-translational modification, plays a pivotal role in regulating the proapoptotic activity of Bad. Recent studies indicate that the ability of Bad to heterodimerize with Bcl-XL and to promote apoptosis depends upon the phosphorylation status of this proapoptotic protein (27, 47). Growth factor (i.e. IL-3 or epidermal growth factor)-induced phosphorylation of Bad at Ser\(^{112}\), Ser\(^{136}\), and Ser\(^{155}\) has been reported to disrupt its proapoptotic function (18–19, 40). Our findings indicate that nicotine mimics growth factors to potently stimulate Bad phosphorylation at these three sites and enhances survival of
pulmonary adenocarcinoma A549 cells (Fig. 1). Other lung cancer cell lines (i.e. H157 and H358) were also tested, and similar results were obtained (data not shown), indicating that nicotine can induce Bad phosphorylation and survival in various lung cancer cells. We discovered previously that nicotine induces Bcl2 phosphorylation in association with increased survival of H69 cells expressing high levels of endogenous Bcl2 (23). Unfortunately, most lung cancer cell lines, including A549 cells, do not express detectable levels of Bcl2, but do express high levels of endogenous Bad (Fig. 1A). Therefore, nicotine-induced survival of A549 cells may occur, at least in part, through phosphorylation of Bad at Ser112, Ser136, and Ser155. Phosphorylation at Ser112 of Bad occurs earlier than Ser136 or Ser155 (Fig. 2A), suggesting that nicotine-induced multi-site Bad phosphorylation may occur in a hierarchical manner. Phosphorylation of Ser112 may facilitate further phosphorylation of Bad at Ser136 and Ser155 sites. However, the signal mechanism and functional significance for this hierarchical phosphorylation remain elusive.

Our results reveal that nicotine-induced multi-site Bad phosphorylation occurs through three signal pathways, including ERKs→Bad at Ser112, PI3K/AKT→Bad at Ser136, and PKA→Bad at Ser155 (Fig. 3 and 4). These results support previous findings that ERKs, AKT, or PKA can function as a Bad Ser112, Ser136, or Ser155 kinase, respectively (26–27, 40, 48). It is known that phosphorylation of Bad on either a single Ser112, Ser136, or Ser155 or at multi-site can inactivate the proapoptotic function of Bad (18–19, 40). PD98059, LY294002, or H89 can potently inhibit nicotine-induced Bad phosphorylation at Ser112, Ser136, or Ser155 in association with decreased cell survival, providing further pharmacological evidence that nicotine-induced multi-site Bad phosphorylation and cell survival may occur through multiple signal pathways involving ERK1/2/Bad, PI3K/AKT/Bad, and PKA/Bad (Fig. 8).

It has been found that high levels of β-adrenergic receptor are expressed in pulmonary adenocarcinoma cells (10). Mounting evidence now indicates that nicotine can function as a β-adrenergic receptor agonist, and its effect is abrogated by...
Propranolol (a β-adrenergic receptor inhibitor; Refs. 11, 49–50). Propranolol was found to block nicotine-induced Bad phosphorylation in A549 cells (Fig. 5), but α-BTX (α7 nAChR specific inhibitor) has no effect (data not shown), suggesting that the β-adrenergic receptor may be the major upstream receptor for nicotine-stimulated Bad phosphorylation in human lung cancer cells. Importantly, inhibition of nicotine-induced Bad phosphorylation by propranolol restores the proapoptotic function of Bad and promotes cell survival.

Phosphorylation on either Ser112 or Ser136 facilitates formation of a complex between Bad and 14-3-3 in the cytosol, blocking its interaction with Bcl-X, at the mitochondrial level (26–27, 48). Further studies indicate that phosphorylation of Ser112, which is located within the center of the Bad BH3 domain, can also directly suppress its proapoptotic function (40). Our findings reveal that nicotine, as a survival agonist, is able to mimic growth factors to functionally inactivate Bad through phosphorylation at these three sites (Figs. 1 and 2). Nicotine-induced multi-site phosphorylation also leads to sequestering Bad from mitochondria in an identical mechanism (Fig. 6). Thus, nicotine may promote survival of human lung cancer cells in a novel mechanism involving multi-site phosphorylation of Bad.

Because Bad is a potent proapoptotic protein that is ubiquitously expressed in both SCLC and non-small cell lung cancer cells (Fig. 1A), targeting Bad phosphorylation may represent a novel therapeutic strategy. Our results indicate that specific knockdown of Bad expression by RNA interference enhances both the survival and chemoresistance of A549 cells (Fig. 7). Importantly, this is a specific effect because nicotine has no additional survival effect on cells in which Bad expression is depleted.

In summary, our studies identify a novel nicotine survival signal transduction pathway that depends on phosphorylation of Bad at Ser112, Ser136, and Ser155 through activation of MAPks ERK1/2, AKT, and PKA (Fig. 8). Nicotine-induced multi-site phosphorylation sequesters Bad from mitochondria, where it then interacts with 14-3-3 in the cytosol, which leads to loss of the apoptotic function of Bad and, hence, enhanced cell survival (Fig. 8). Because Bad can function as a target of nicotine in human lung cancer cells, it’s use may help to develop novel therapeutic strategies for treatment of patients with lung cancer by blocking the nicotine-activated multiple Bad upstream signal pathways.

REFERENCES

1. Hecht, S. S. (1999) J. Natl. Cancer Inst. 91, 1194–1210
2. American Cancer Society (2003) Cancer Facts and Figures, pp. 13–14, American Cancer Society Inc., Atlanta, GA
3. Schuller, H. M. (2002) Nat. Rev. Cancer 2, 455–463
4. Shopland, D. R. (1995) Environ. Health Perspect. 103, 131–142
5. Karnath, B. (2002) Am. J. Med. 112, 399–405
6. Minna, J. D. (2003) J. Clin. Invest. 111, 31–33
7. Heusch, W. L., and Maneckepp, R. (1998) Carcinogenesis 19, 551–556
8. Iser, V., and Bertrand, D. (2001) FEBS Lett. 504, 118–125
9. Garnier, M., Lamarc, M., Tonon, M. C., and Vauduy, H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11743–11747
10. Park, P. G., Merriman, J., Orloff, M., and Schuller, H. M. (1995) Cancer Res. 55, 3594–3598
11. Suemaru, K., Kawasaki, H., Oishi, R., Gomita, Y., and Tanizaki, Y. (1997) Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 571–575
12. Littez, G. P., Hess, J. L., Sentman, C. L., and Korsmeyer, S. J. (1995) Blood 86, 1255–1260
13. Cory, S., and Adams, J. M. (2000) Nat. Rev. Cancer 2, 647–656
14. Sartorius, U. A., and Krammer, P. H. (2002) Int. J. Cancer 97, 584–592
15. Kelekser, A., and Thompson, C. B. (1998) Trends Cell Biol. 8, 324–330
16. Green, D. R., and Evan, G. I. (2002) Cancer Cell 1, 19–30
17. Huang, D. C., and Strasser, A. (2000) Cell 100, 639–642
18. Hirai, I., and Wang, H. G. (2001) Biochem. J. 359, 345–352
19. Tan, Y., Demeter, M. R., Ruan, H., and Comb, M. J. (2000) J. Biol. Chem. 275, 25865–25869
20. Blessmann, A. (2002) Dev. Cell 3, 607–608
21. Datta, S. R., Katev, A., Hu, L., Petros, A., Fesik, S. W., Yaffe, M. B., and Greenberg, M. E. (2000) Mol. Cell 6, 41–51
22. Zha, J., Harada, H., Yang, E., Ito, K., and Korsmeyer, S. J. (1996) Cell 87, 619–628
23. Mai, H., May, Jr., W. S., Gao, F., Jin, Z., and Deng, X. (2003) J. Biol. Chem. 278, 1886–1891
24. Ikegaki, N., Katsumata, M., Minna, J., and Tsujimoto, Y. (1994) Cancer Res. 54, 6–8
25. West, K. A., Brognard, J., Clark, A. S., Linnola, I. R., Yang, X., Swain, S. M., Harris, C., Belinsky, S., and Denus, P. A. (2003) J. Clin. Invest. 111, 81–90
26. Scheid, M. P., Schubert, K. M., and Duronio, V. (1999) J. Biol. Chem. 274, 31108–31113
27. Datta, S. R., Dudev, H., Tao, X., Masters, S., Hu, G., Goh, Y., and Greenberg, M. E. (1997) Cell 91, 231–241
28. Hayakawa, J., Onohata, M., Kurachi, H., Kanda, Y., Hisamoto, K., Nishio, Y., Adachi, K., Tasaka, K., Kanzaki, T., and Murata, Y. (2000) Cancer Res. 60, 5988–5994
29. Deng, X., Ruvolo, P., Carr, B., and May, Jr., W. S. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1578–1583
30. Ito, T., Deng, X., Carr, B., and May, Jr., W. S. (1997) J. Biol. Chem. 272, 11671–11673
31. Goechi, B. S., Hsiao, K., Tereba, A., and Goechi, S. A. (1995) Anal. Biochem. 235, 10–17
32. Deng, X., Xiao, L., Lang, W., Gao, F., Ruvolo, P., and May, Jr., W. S. (2001) J. Biol. Chem. 276, 23681–23688
33. Antonsson, B., Montessuit, S., Sanchez, B., and Martinou, J. C. (2001) J. Biol. Chem. 276, 16115–16123
34. Ikem, E., Fiedler, R., Parten, R. G., and Simons, K. (1995) FEBS Lett. 358, 273–277
35. Gross, A., McDonnell, J. M., and Korsmeyer, S. J. (1999) Genes Dev. 13, 1199–1211
36. Datta, S. R., Ranger, A. M., Lin, M. Z., Sturgill, J. F., Ma, Y. C., Cowan, C. W., Dikkes, P., Korsmeyer, S. J., and Greenberg, M. E. (2002) Dev. Cell 3, 631–641
37. Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, F. (1986) Biochem. Biophys. Res. Commun. 135, 397–402
38. Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 27489–27494
39. Vlahos, C. J., Matter, W. F., Hui, K. Y., and Brown, R. F. (1994) J. Biol. Chem. 269, 5241–5248
40. Zhao, X., Liu, Y., Payne, G., Lutz, R. J., and Thomas Chittenden, T. (2000) J. Biol. Chem. 275, 25046–25051
41. Schuller, H. M., Thihof, P. K., Williams, M., and Plummer, H. (1999) Cancer Res. 59, 4510–4515
42. Couturier, S., Bertrand, D., Matter, J. M., Hernandez, M. C., Bertrand, S., Millar, N., Valera, S., Barkas, T., and Ballivet, M. (1990) Neuron 5, 847–856
43. Perissin, L., Rapozzi, V., Zorzetti, G., and Giralidi, T. (1996) Anticancer Res. 16, 3409–3413
44. Elbashir, S. M., Harborth, J., Weber, K., and Tuschl, T. (2002) Methods 26,
45. Yang, E., Zha, J., Jockel, J., Boise, L. H., Thompson, C. B., and Korsmeyer, S. J. (1995) Cell 80, 285–291
46. Lutz, R. J. (2000) Biochem. Soc. Trans. 28, 51–56
47. Wang, H. G., Pathan, N., Ethell, I. M., Krajewski, S., Yamaguchi, Y., Shibasaki, F., McKeon, F., Bobo, T., Franke, T. F., and Reed, J. C. (1999) Science 284, 339–343
48. Fang, X., Yu, S., Eder, A., Mao, M., Bast, R. C., Boyd, D., and Mills, G. (1999) Oncogene 18, 6635–6640
49. Gaddek-Michalska, A., Bugajski, J., Bugajski, A. J., and Glod, R. (2002) J. Physiol. Pharmacol. 53, 275–287
50. Uchida, S., Kawashima, K., and Lee, T. J. (2002) Auton. Neurosci. 96, 126–130
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