p38 Mitogen-activated Protein Kinase-independent Induction of gadd45 Expression in Nerve Growth Factor-induced Apoptosis in Medulloblastomas*

We describe a novel nerve growth factor (NGF)-signaling pathway leading to gadd45 induction that is independent of JNK and p38 MAPK. We used cDNA arrays representing 588 genes to investigate the role of differential gene expression in NGF-mediated pleiotropic responses. We compared the gene expression profiles obtained from MED283-TrkA cells undergoing NGF-induced apoptosis to PC12 cells undergoing NGF-induced differentiation. An early and specific transcriptional target of NGF in MED283-TrkA cells was the DNA-damage-inducible gene gadd45. Its magnitude of induction directly correlated with the magnitude of apoptosis in MED283 clones transfected with mutant TrkA receptors. Although gadd45 has been implicated in stress response signaling, in vitro kinase assays indicated that NGF neither activated c-Jun NH2-terminal kinase (JNK) nor p38 mitogen-activated protein kinase (MAPK). Furthermore, the p38 MAPK inhibitor SB203580 (20 μM) failed to prevent NGF-induced apoptosis and NGF-induced gadd45 expression. These results suggest that differential regulation of gadd45 expression possibly through BRCA1 may be a potential mechanism whereby NGF regulates pleiotropic responses.

Nerve growth factor (NGF) belongs to a family of closely related growth factors known as neurotrophins (1, 2). The neurotrophins bind to and activate specific receptor tyrosine kinases of the Trk family (1, 2). TrkA, TrkB, and TrkC are respectively the receptors for NGF, BDNF, and NT-3 (1, 2). In addition, NT-4/5 also binds to the TrkB receptor (1, 2). The mechanisms of Trk receptor activation and downstream signal transduction are similar to those employed by other tyrosine kinase receptors (3). The ultimate effect of such signaling is to induce phosphorylation and activation of transcription factors in the nucleus and to initiate new programs of gene expression (4, 5). Genes regulated by growth factors such as NGF are broadly classified as either immediate early genes, whose mRNA induction does not require protein synthesis, or late response genes (5). Many immediate early genes have been shown to encode transcription factors, including c-fos, Fra-1, fos-B, c-jun, jun-B, jun-D, NGFI-A (also known as EGR1, zif/268, and Krox-24), NGFI-B (nur77), and c-myc (5). By directing specific programs of late gene expression, the induction of these proteins could coordinate long term responses to growth factor stimulation. In the rat pheochromocytoma cell line PC12, a model for NGF signaling, a number late response genes to NGF have been identified (5), including: transthyretin, perseprerin, SGC10, neurofilament (NF)-L, NF-M, 41A, 41C, neuronal cell adhesion molecule (NCAM), and Vgf.

In addition to their trophic effect on peripheral sympathetic and sensory neurons, neurotrophins trigger other biological responses in a variety of other cell types (1). For example, deletion of the trkA gene in mice results in atrophy and a decreased number of cholinergic neurons in the central nervous system (6–9). Outside of the nervous system, TrkA has been identified in a variety of bone marrow-derived cell types, including monocytes, lymphocytes, and basophils (10, 11). NGF and other neurotrophins also play important roles in injury response, and they have been implicated as oncopgenes and tumor suppressors in various human tumors (12–15). Indeed, high expression of trkA and trkC correlates with a favorable prognosis in neuroblastoma and medulloblastoma, respectively (14–16).

The molecular basis underlying neurotrophin response pleiotropism is not well understood, and it may be transcriptional or post-transcriptional or a combination of both. Iyer et al. (17) explored the effects of serum on fibroblast gene expression using cDNA arrays. Fambrough et al. (18) explored the question of dependence of immediate early gene induction on the various signaling pathways activated by platelet-derived growth factor β receptor using oligonucleotide arrays. They found that various signaling pathways exerted broadly overlapping effects on immediate early gene induction (18).

We previously reported that overexpression of TrkA in MED283 cells results in massive NGF-induced apoptosis (19). This contrasts with PC12 cells, which differentiate in response to NGF treatment (20). Surprisingly, mutations to TrkA, which hindered NGF-induced differentiation in PC12 cells, also abolished NGF-induced apoptosis in MED283 cells (21). Here, we compared the gene expression profiles obtained from MED283-TrkA cells undergoing NGF-induced apoptosis to PC12 cells undergoing NGF-induced differentiation. We provide novel evidence that NGF-induced apoptosis is linked to the induction of gadd45 through a p38 MAPK-independent mechanism.
EXPERIMENTAL PROCEDURES

Materials—Mouse NGF (2.5 S, final concentration: 100 ng/ml) was obtained from Collaborative Biomedical Products, PD89059 (final concentration: 50 µg/ml) was obtained from New England Biolabs, SB203580 (final concentration: 20 or 40 µg/ml) was obtained from Calbiochem, and cycloheximide (final concentration: 300 µg/ml) was obtained from Sigma.

Cell Culture—PC12 cells were cultured in 10% CO2 on collagen-coated plates in RPMI 1640 supplemented with 5% fetal bovine serum, 10% horse serum, 50 units/ml penicillin, and 50 µg/ml streptomycin. MED283 cells expressing wild-type and mutant TrkA have been described elsewhere (21) and were cultured in 10% CO2 in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 units/ml penicillin, 50 µg/ml streptomycin, and 2 mM G418.

Filter Array Hybridization and Analysis—Total RNA was isolated using the Totally RNA kit from Ambion according to the manufacturer’s instructions, and poly(A)+ RNA was isolated using the PolyATract mRNA isolation system from Promega according to the manufacturer’s instructions. Gene expression was analyzed using the human and rat Atlas cDNA expression arrays (CLONTECH Laboratories) containing 588 distinct human or rat transcripts according to the manufacturer’s instructions. Washed membranes were exposed to a phosphor screen for 3 days and scanned using a Storm Scanner (Molecular Dynamics) at 50-µm pixel resolution. Autoradiographic intensity for each gene was analyzed using the ImageQuant software (Molecular Dynamics). Before relative changes in gene expression level were determined, the raw expression data were normalized to the ratio of the median intensities of the NGF-treated and untreated arrays. Based on our analysis, a 3-fold or more change in intensity corresponds to roughly 2.0 S.D., our threshold for significant change. Candidate genes were manually screened to exclude points contaminated by locally high background noise. Genes were clustered hierarchically into groups on the basis of the similarity of their expression profiles as described (22).

Northern Blots—Total RNA was isolated using the Totally RNA kit from Ambion according to the manufacturer’s instructions. Northern blots were performed using the standard techniques described elsewhere (21) and were cultured in 10% CO2 in RPMI 1640 supplemented with 5% fetal bovine serum.

RESULTS

Messenger RNA isolated from MED283-TrkA cells and PC12 cells at 0, 1, 6, and 24 h after NGF treatment were radiolabeled and hybridized to cDNA arrays with 588 known transcripts. RNA from the MED283-TrkA cell line and the PC12 cell line were hybridized against their respective human and rat arrays. Those genes of special interest not present in both arrays were further characterized by Northern blots containing homologous human and rat cDNAs. Based on reproducibility experiments, we determined differences in the relative expression level of 3-fold or more to be potentially significant. Diverse temporal profiles of gene expression could be seen among the 600 cDNAs surveyed (Fig. 1).

Transcripts were clustered hierarchically into groups on the basis of the similarity of their expression profiles (Fig. 2). Genes that encode transcription factors dominate early response to NGF stimulation. A total of 7 genes were induced within 1 h after NGF stimulation in the MED283-TrkA and PC12 cells combined, i.e. arc, c-fos, c-jun, egr1, fra-1, and nur77, and they all have been shown to be NGF-regulated (5). Consistent with the behavior of immediate early genes, the increased expression of all these genes was transient, and most returned to base line 6 h after NGF treatment. Other NGF target genes identified by array hybridization experiments are listed in Tables I and II.

In PC12 cells, vgf, a previously described NGF-regulated transcript (25), was induced at 6 h. Studies from knockout mice suggest that Vgf plays a critical role in the control of energy homeostasis (26). In MED283-TrkA cells, two genes, BTEB2 and gadd45, were induced at 6 h. BTEB2 is a zinc finger, GC-box binding transcription factor that is most highly expressed in smooth muscle tissues (27). Genes specifically regulated by BTEB2, and the physiologic function of BTEB2 is poorly understood. However, the induction of BTEB2 has been implicated in cellular responses to vascular injury models (28). Although BTEB2 was also up-regulated in PC12 cells (Fig. 3A), gadd45 induction was restricted only to the MED283-TrkA cells. gadd45 encodes a stress- and DNA-damage-responsive gene, initially characterized as genotoxic stress responsive (29,
and more recently suggested to also mediate activation of JNK and p38 MAPK (31–33). gadd45 induction could be inhibited by the protein synthesis inhibitor cycloheximide (data not shown), thus distinguishing it from immediate early genes. The significance of gadd45 induction will be further explored later on in this paper.

24 h after NGF treatment, ID-1h and transducer of erbB-2 (TOB), were induced in MED283-TrkA cells, whereas RACH1 and CUT were suppressed. Little is known about the function of ID-1h, although this family of helix-loop-helix transcription factors may be important in cell cycle progression (34). In contrast, TOB, through its interaction with erbB-2 and its homology to the anti-proliferative protein B-cell translocation gene 1 (BTG1), has been implicated as a negative regulator of cell cycling (35). The significance of RACH1 and CUT suppression is unclear. RACH1 encodes a urea transporter (36), whereas CUT encodes a putative repressor of developmentally regulated gene expression (37). Its down-regulation would be consistent with an attempt by NGF-treated cells to undergo differentiation. Of note, we did not detect in MED283-TrkA cells significant changes in the expression of apoptosis-related genes, e.g. members of the Bcl-2 and caspase families. This would be consistent with a post-transcriptional role for these proteins in apoptosis. In PC12 cells, we detected significant gene expression changes at 24 h in transin, neuromodulin (GAP43), neuronatin, and GST and DCC (deleted in colon cancer. All of these genes except DCC have been reported to be NGF-responsive (38–41), and all including DCC have been implicated in maintaining neuronal phenotype (42).

The induction of gadd45 in MED283-TrkA cells validates the genotoxicity of NGF via TrkA, and it provides an early marker of NGF-induced apoptosis. At least two distinct pathways leading to the induction of gadd45 have been described. Ionizing radiation triggers the ATM- and p53-dependent induction of gadd45 (30, 43). Other stimuli, including ultraviolet (UV) irradiation, treatment with alkylating agents, and serum starvation, lead to the induction of gadd45 by a p53-independent pathway (44). We examined the effect of NGF treatment on p53 expression in both MED283-TrkA and PC12 cells. Although NGF has been shown to induce the expression of p53 in PC12 cells several days after NGF treatment (45), at 6 h, when gadd45 induction was observed, we did not observe any change in p53 expression in either the MED283-TrkA or the PC12 cell line (Fig. 3A). Contrary to this, the expression of another known p53 target, p21 (WAF1), was induced by NGF at 6 h (Fig. 3A). These results agree with previously published reports indicating that NGF induces p21 independently of the p53 pathway (46, 47), and although p21 is an important mediator of differentiation, it does not appear to be a regulator of apoptosis (45, 48).

To better characterize the time course of gadd45 induction, real-time PCR was carried out on cDNA reverse-transcribed from MED283-TrkA cells using gadd45-specific primers and probes (Fig. 3B). Our PCR data were qualitatively similar to our microarray data, although quantitatively it showed that the induction of gadd45 transcript peaked earlier and at 2 h and also subsided earlier, at 6 h, after NGF treatment. This difference may be technical, secondary to differences in the employed cell culture or treatment conditions. Nevertheless, the PCR results confirm our microarray and Northern blot
data, and they indicate that gadd45 is an early transcriptional target of NGF signaling in the MED283-TrkA cells.

To further examine the specificity of increased gadd45 expression with NGF-induced apoptosis, we quantitated its induction in several MED283 clones stably expressing mutant TrkA receptors (21). Consistent with our previously study (21), treatment of these clones with NGF demonstrated a critical requirement for Trk kinase activity (K538N) as well as at least one of two functional tyrosine sites mediating SHC (Y490F) or phospholipase Cγ1 (PLC-γ1) (Y785F) binding in NGF-induced apoptosis (Fig. 4A). Indeed, we found that the induction of gadd45 mRNA among these clones closely paralleled their ability to undergo apoptosis (Figs. 4, B and C).

The observation that NGF induces expression of gadd45 and triggers apoptosis in MED283-TrkA cells is noteworthy in light of the recent discovery that gadd45 may interact with the stress-responsive MTK1/MEKK4, leading to JNK/p38 MAPK-dependent apoptosis (33). Therefore, we next characterized their activities after NGF treatment by in vitro kinase assays on immunoprecipitated kinases. Although NGF induced a rapid and sustained activation of ERK activity in both PC12 and MED283-TrkA cells (Fig. 5A), we did not detect any increase in p38 MAPK and JNK kinase activity after NGF treatment in PC12 cells and MED283-TrkA cells, even though osmotic stress (0.4 M NaCl) led to their robust activation (Fig. 5, B and C).
We also examined whether the application of the p38 MAPK-specific inhibitor SB203580 can block NGF-induced apoptosis or *gadd45* induction or both. We found that SB203580 (20 μM) did not prevent cells from NGF-induced apoptosis (Fig. 6A) even though it inhibited p38 MAPK kinase activation after osmotic shock (0.4 M NaCl) (Fig. 6B).

**Fig. 3.** *gadd45* induction by NGF in MED283-TrkA cells. A, selective *gadd45* induction by NGF in MED283-TrkA cells confirmed by Northern blots. Total cellular RNA from MED283-TrkA cells and PC12 cells untreated or treated with NGF for 6 h were analyzed using probes against the indicated candidate targets. B, time course of *gadd45* induction in MED283-TrkA cells by real-time PCR. Real-time PCR reactions were carried out using *gadd45* and β2-microglobulin-specific primers and Taqman probes. Data from four independent experiments were normalized using control samples and β2-microglobulin values to give relative units of mRNA induction. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

**Fig. 4.** Point mutations in the TrkA receptor that abolish apoptosis also prevented *gadd45* induction. A, MED283 cells expressing wild type (WT), K538N (kinase inactive), Y490F (SHC-deficient), Y785 (PLC-γ1-deficient), Y490/785 (SHC- and PLC-γ1-deficient) TrkA constructs were treated with NGF for 72 h. Fragmented DNA were extracted and resolved by agarose gel electrophoresis. B, total cellular RNA from MED283 cells expressing mutant and wild-type TrkAs untreated (−) or treated with NGF for 6 h (+) were analyzed using probes against the *gadd45* or *GAPDH* (top). The panels have been rearranged to match those in panel A. *gadd45* expression level of NGF-treated (white bars) and untreated (block bars) samples were quantitated using a PhosphorImager and normalized relative to *GAPDH* expression (bottom). Data are representative of two independent experiments. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.
Although SB203580 at 20 μM did not completely inhibit p38 MAPK activation, neither did higher concentrations (up to 40 μM), although higher concentrations were cytotoxic (data not shown).
shown). We had previously shown that NGF-induced apoptosis occurred through Ras but was independent of the ERK/MAPK pathway (21). Next we determined whether ERK or p38 MAPK activation was required for NGF-mediated gadd45 induction. We examined the effects of SB203580 (20 μM) and MEK1 inhibitor PD98059 (50 μM) treatments on gadd45 expression by Northern blots. Neither SB203580 nor PD98059 altered the induction of gadd45 at 6 h after NGF treatment (Fig. 6C, data not shown). These results suggest that p38 MAPK and ERK pathways do not participate in gadd45 induction.

**DISCUSSION**

In this paper, we sought to understand the gene expression events leading to NGF-induced apoptosis versus differentiation in MED283-TrkA versus PC12 cells. We examined a total of 588 genes representing roughly 2% of all genes estimated to be present in the human genome (49, 50). Analysis of the temporal pattern of gene expression profiles after NGF treatment revealed that genes with similar functional roles tended to share similar gene regulatory mechanisms. For example, immediate early genes underwent rapid and transient induction after NGF treatment. In this category, 4 immediate early genes (0.7%) were induced in PC12 cells and 4 immediate early genes (0.7%) were induced in MED283-TrkA cells. These ratios were similar to the results of Fambrough et al. (18), who reported that the tyrosine kinase receptor platelet-derived growth factor receptor-β induced 66 immediate early genes out of 6000 genes (~1%). Our ratios are lower most likely because we used a higher threshold for classifying significant change in gene expression. Although this survey is far from comprehensive, the similarity of the immediate early genes induced in MED283-TrkA and PC12 cells would suggest that immediate early gene induction occurred via common signaling pathways (18). Consistent with this hypothesis, both cell lines responded to NGF by activation of ERK, PLC-γ, and phosphatidylinositol 3-kinase pathways, whereas neither cell line showed significant activation of p38 MAPK and JNK pathways (21). This would suggest that NGF response specificity is encoded downstream from immediate early gene activation, presumably through epigenetic changes in chromatin structure, locus accessibility, and DNA methylation.

A second wave of gene expression changes was detected starting 6 h after NGF treatment. Although in PC12 cells these late response genes were all associated with neuronal differentiation, in MED283-TrkA cells they were primarily involved in cell cycling and growth arrest. The difference in induced late response genes suggests that PC12 cells have already committed to neuronal differentiation, whereas MED283-TrkA cells exist primarily in an undifferentiated state. Alternatively, MED283-TrkA cells may have also committed to neuronal differentiation, but other factors such as Sonic Hedgehog are required to unlock differentiation pathways (51). If this were the case, the other factors would have to activate sufficiently distinct signaling pathways and immediate early genes so that a different program of late response genes is activated. Either way, NGF treatment induced many cell cycle arrest genes (gadd45, p21 WAF1/CIP1, TOB) in MED283-TrkA cells despite persistently high levels of c-Myc and ID-1h expression (52), an observation that supports conflicting growth control signals as a mechanism of NGF-induced apoptosis (53).

Another major finding of this study is the early and sustained induction of gadd45 in MED283-TrkA cells undergoing apoptosis. gadd45 induction occurred significantly before the onset DNA fragmentation, which was not seen until at least 24 h after NGF treatment (19). Because gadd45 induction is associated with exposure to genotoxic stresses such as ionizing irradiation and alkylating agents, its induction in MED283-TrkA cells may represent a marker of DNA damage. Alternatively, gadd45 has recently been suggested to play a more direct role in apoptosis through activating the stress-activated protein kinase pathways, p38 MAPK and JNK (33). Based on our results, NGF treatment failed to activate p38 MAPK, and it also had no effect on JNK1 and JNK3 kinase activities. Moreover, the p38 MAPK inhibitor SB203580 neither inhibited NGF-induced apoptosis nor NGF-induced gadd45 expression. In contrast, Kim et al. (16) report that high concentrations of SB203580 (25–75 μM) partially blocked NT-3-induced apoptosis in Daoy-TrkC cells (16). In addition, they observed that NGF led to transient phosphorylation of p38 MAPK, peaking at 1 h (16). This transient activation of p38 MAPK was not seen in MED283-TrkA cells. The reason for this discrepancy is presently unclear and may be cell line-specific (21). Nevertheless, the induction of gadd45 in the absence of p38 MAPK or JNK activation suggests the presence of additional regulatory pathways.

Both p53-dependent and independent pathways leading to gadd45 induction have been described. Our results indicate that NGF induces gadd45 through a p53-independent mechanism. In this respect, the breast and ovarian cancer susceptibility gene product BRCA1 has been shown to transactivate the gadd45 promoter through a p53-independent manner (54, 55), and it may be a potential mechanism whereby NGF induces gadd45 expression or apoptosis or both. Indeed, restoration of BRCA1 in BRCA1-deficient tumor cell lines triggers apoptosis through gadd45 (56). Consistent with this line of reasoning, both BRCA1-mediated gadd45 induction and NGF-induced apoptosis have been shown to be critically dependent on phosphatidylinositol 3-kinase activity (21, 57), suggesting a common upstream pathway for both events. Although BRCA1 is best characterized for its ability to inhibit the proliferation of mammary epithelial cells, BRCA1 is highly expressed in the proliferating mid- and hindbrain and BRCA1-deficient mice die in utero with severe neural tube abnormalities (58, 59). How may BRCA1 selectively regulate gadd45 in NGF-induced apoptosis versus differentiation? Some possible mechanisms may include differential regulation of BRCA1 expression level, differences in the mutational/functional state of BRCA1 proteins, and differences in the activities of its upstream signaling pathways. These hypotheses will have to be experimentally tested. Interestingly, the epidermal growth factor receptor overexpressing A431 epidermoid carcinoma cell line also undergoes gadd45 induction and apoptosis after epidermal growth factor treatment (60). It is tempting to speculate the existence of a “shunt” pathway that senses growth factor overstimulation via BRCA1, which then leads to gadd45 activation, cell cycle arrest, and apoptosis.

In summary, we demonstrated for the first time a novel pathway of NGF signaling involving gadd45 that is independent of JNK or p38 MAPK. Although the precise mechanism by which NGF induces gadd45 is currently unknown, its differential expression may be a potential mechanism whereby NGF regulates pleiotropic responses. Additional studies of these signaling pathways may provide insights into mechanisms that regulate the normal development of the nervous system as well as the induction and progression of medulloblastomas and other pediatric brain tumors.

Acknowledgments—We gratefully acknowledge R. M. Stephens and D. R. Kaplan for TrkA mutant constructs, M. B. Eisen for the cluster analysis and display software, C. D. Paige for technical assistance, and G. M. Brodeur and J. Eberwine for helpful discussion.

**REFERENCES**

1. Barbacid, M. (1995) *Curr. Opin. Cell Biol.* 7, 148–155
2. Bothwell, M. (1995) *Annu. Rev. Neurosci.* 18, 223–253
3. van der Geer, P., Hunter, T., and Lindberg, R. A. (1994) *Annu. Rev. Cell Biol.*
10. Levi-Montalcini, R., Dal Toso, R., della, V. F., Skaper, S. D., and Leon, A.
12. Bongarzone, I., Pierotti, M. A., Monzini, N., Mondellini, P., Manenti, G.
11. Levi-Montalcini, R., Skaper, S. D., Dal Toso, R., Petrelli, L., and Leon, A.
13. Mitra, G., Martin-Zanca, D., and Barbacid, M. (1987)
14. Nakagawara, A., Arima-Nakagawara, M., Scavarda, N. J., Azar, C. G., Cantor,
23. Giasson, B. I., and Mushynski, W. E. (1996)
26. Hahm, S., Mizuno, T. M., Wu, T. J., Wisor, J. P., Priest, C. A., Kozak, C. A.,
25. Salton, S. R., Ferri, G. L., Hahm, S., Snyder, S. E., Wilson, A. J., Possenti, R.,
24. Takenaka, K., Moriguchi, T., and Nishida, E. (1998)
20. Greene, L. A., and Tischler, A. S. (1976)
19. Muragaki, Y., Chou, T. T., Kaplan, D. R., Trojanowski, J. Q., and Lee, V. M.
21. Chou, T. T., Trojanowski, J. Q., and Lee, V. M. (2000)
J. Biol. Chem.
22. Eisen, M. B., Spellman, P. T., Brown, P. O., and Botstein, D. (1998)
Proc. Natl. Acad. Sci. U. S. A. 95, 1366–1466
27. Sogawa, K., Imataka, H., Yamasaki, Y., Kusume, H., Abe, H., and Fujii-
31. Gerwins, P., Blank, J. L., and Johnson, G. L. (1997) J. Biol. Chem. 272,
8288–8295
32. Takekawa, M., Pousas, F., and Saito, H. (1997) EMBO J. 16, 4973–4982
33. Takekawa, M., and Saito, H. (1998) Cell 95, 521–530
34. Hara, E., Yamaguchi, T., Nogawa, H., Ide, T., Campisi, J., Okayama, H., and
35. Matsuda, S., Kawamura-Tsuzuku, J., Obami, M., Yoshida, M., Eimi, M.,
36. Nakamura, T., Onda, M., Yoshida, Y., Nishiyama, A., and Yamamoto, T.
(1996) Oncogene 12, 705–713
38. Shayanl, C., Steel, A., and Hediger, M. A. (1996) J. Clin. Invest. 98,
39. Meiri, K. F., Pfenninger, K. H., and Willard, M. B. (1986)
Proc. Natl. Acad. Sci. U. S. A. 83, 3577–3581
40. Joseph, R., Tsang, W., Duv, D., Nelson, K., and Edvardson, K. (1996) Brain Res.
73, 32–38
41. Tsur, E. K. M., Qureshi, M. M., Ijaz, M. K., Galadari, S. H., and Raza, H. (2000)
Int. J. Oncol. 16, 1043–1048
42. Shu, T., Valentine, K. M., Seaman, C., Cooper, H. M., and Richards, L. J.
(2000) J. Exp. Med. 194, 201–212
43. Kanada, M., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V.,
44. Hollander, M. C., Alamo, I., Jackman, J., Wang, M. G., McBr ide, O. W., and
45. Poluha, W., Schonhoff, C. M., Harrington, K. S., Lacyhyan, M. B., Crosby,
46. Yan, G. Z., and Ziff, E. B. (1997) J. Neurosci. 17, 6122–6132
47. van Grunsven, L. A. B., Bullo, N., Savatier, P., Thomas, A., Urdiales, J. L., and
48. Erhardt, J. A., and Pittman, R. N. (1998) Oncogene 16, 443–451
49. Venter, J. C., Adams, M. D., and Myers, R. W., et al. (2001) Science 291,
1301–1315
50. Lander, E. S., Linton, L. M., Birren, B., et al. (2001) Nature 409, 860–921
51. Weinberger-Raya, R. J., and Scott, M. P. (1999) Neuron 24, 103–114
52. Bigner, S. H., Friedman, H. S., Vogelstein, B., Oakes, W. J., and Bigner, D. D.
(1990) Cancer Res. 50, 2347–2350
53. Dihenedetto, A. J., and Pittman, R. N. (1996) Perspect. Dev. Neurobiol. 3,
111–120
54. Jin, S. Z., Zhao, H., Fan, F., Blanck, P., Fan, W., Colchagia, A. B., Forncate, A. N.,
and Zhan, Q. (2000) Oncogene 19, 4505–4507
55. MacLachlan, T. K., Samanidaram, K., Saggas, M., Shifman, Y., Muschel,
R. J., Cowan, K. H., and El Deiry, W. S. (2000) J. Biol. Chem. 275,
2777–2785
56. Harkin, D. P., Bean, J. M., Miklos, D., Song, Y. H., Truong, V. B., Englert,
Christian, P. C., Ellisen, L. W., Maeswaran, S., Oliner, J. D., and Haber,
D. A. (1999) Cell 97, 575–586
57. Altiok, S., Kurabayashi, M., Shimosura, Y., Kawai-Kawase, K., Hoshino,
Manabe, T., Watanabe, M., Akawaka, M., Kuro, Suzuki, T., Yazaki, Y., and
Nagai, R. (1999) Circ. Res. 85, 182–191
58. Fornace, A. J. J., Alamo, I. J., and Hollander, M. C. (1998) J. Biol. Chem.
97, 8800–8804
59. Gerwins, P., Blank, J. L., and Johnson, G. L. (1997) J. Biol. Chem. 272,