Aph2, a Protein with a zf-DHHC Motif, Interacts with c-Abl and Has Pro-apoptotic Activity*

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The abbreviations used are: SH, Src homology; ER, endoplasmic reticulum; aa, amino acids; Aph2, Abl-philin 2; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling; X-gal, 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside; MBP, maltose-binding protein.

C-Abl is a non-receptor tyrosine kinase implicated in DNA damage-induced cell death and in growth factor receptor signaling. To further understand the function and regulation of c-Abl, a yeast two-hybrid screen was performed to identify c-Abl-interacting proteins. Here we report the identification of Abl-philin 2 (Aph2), encoding a novel protein with a unique cysteine-rich motif (zf-DHHC) and a 53-amino acid stretch sharing homology with the creatine kinase family. The zf-DHHC domain is highly conserved from yeast to human. Two proteins containing this motif, Akrlp and Er2lp, have been characterized in Saccharomyces cerevisiae, both implicated in signaling pathways. Deletion analysis by two-hybrid assays revealed that the N-terminal portion of Aph2 interacts with the C terminus of c-Abl. Aph2 was demonstrated to interact with c-Abl by co-immunoprecipitation assays. Aph2 is expressed in most tissues tested and is localized in the cytoplasm, mainly in the endoplasmic reticulum (ER). The sequences required for ER location reside in the N terminus and the zf-DHHC motif of Aph2. It has been reported that a portion of c-Abl is localized in the ER. We demonstrate here that Aph2 and c-Abl are co-localized in the ER region. Over-expression of Aph2 leads to apoptosis as justified by TUNEL assays, and the induction of apoptosis requires the N terminus. Co-expression of c-Abl and Aph2 had a synergistic effect on apoptosis induction and led to a decreased expression of both proteins, suggesting either that these two proteins are mutually down-regulated or that cells expressing both c-Abl and Aph2 rapidly disappeared from the culture. These results suggest that Aph2 may be involved in ER stress-induced apoptosis in which c-Abl plays an important role.

C-Abl is a ubiquitously expressed non-receptor tyrosine kinase. This protein has Src homology domains (SH1, SH2, and SH3) at the N terminus and a DNA binding domain, an actin-binding domain, three nucleus localization signals, and a proline-rich motif at the C terminus (reviewed in Refs. 1 and 2). The C terminus is essential for c-Abl function and is a unique feature not found in other Src family members. c-Abl is localized in both the nucleus and the cytoplasm. Movement between these two compartments may play an important role in regulating c-Abl function (3, 4). c-Abl is activated by DNA damage, oxidative stress, cell adhesion to extracellular matrix, growth factors, and Src family kinases (reviewed in Refs. 1 and 2). However, the physiological function of c-Abl is not well understood. Mice lacking c-Abl showed perinatal death, runtedness, reduced fertility, lymphopenia, and osteoporosis (5–7). Arg (Abl-related protein), a c-Abl homologue, has a similar structure (8). Arg(−/−) mice develop normally, whereas embryos deficient in both Abl and Arg suffer from defects in neurulation and die before 11 days postcoitum (9).

C-Abl plays an important role in apoptosis (1). Ectopic expression of c-Abl in fibroblasts causes apoptosis in a p53-independent manner (10, 11). Epithelial cells stably expressing a mutant c-Abl without kinase activity (K290R) are resistant to apoptosis induced by either anti-metabolite 1-b-D-arabinofuranosylcytine (ara-C) or ionizing radiation (12, 13). Furthermore, c-Abl-null mouse embryonic fibroblasts showed resistance to apoptosis induced by the same treatments (14). These findings provide evidence for a pro-apoptotic function of c-Abl. On the other hand, c-Abl has also been demonstrated to have an anti-apoptotic effect. Pre-B lymphocytes isolated from c-Abl-null mice are more sensitive to IL-7 withdrawal or glucocorticoid-induced apoptosis (15). Osteoblasts from c-Abl-null mice also are more sensitive to free radical-induced cell death.5 In these two cell types, c-Abl apparently protects cells from cell death. Furthermore, the oncogenic form of c-Abl (BCR-ABL) is anti-apoptotic, and the anti-apoptotic activity could be due to the cytoplasmic localization of BCR-ABL (16).

Many c-Abl-interacting proteins have been identified (reviewed in Ref. 17). Some are prominent substrates for c-Abl (e.g. Abi1/Abi2, Crk1/CrkL, RNA polymerase II, p62dok, and p73), and some of them are not (e.g. p53, Rb, 3BP-1/3BP-2, AAP1, ATM and DNA-PK, PAG) (18). Some of the interacting proteins, such as PAG and Rb, were found to negatively regulate c-Abl kinase activity and function, whereas others, such as RFXI, an EP-DNA-binding protein that potentiates the kinase activity when bound to c-Abl, were found to be positive regulators for c-Abl (19).

In this study we identified a novel c-Abl-interacting protein,
Aph2, by a two-hybrid screen. The protein has a cysteine-rich domain that is conserved in many proteins from yeast to mammals. Aph2 is ubiquitously expressed in tissues tested and is mainly localized in the ER of cells. When ectopically expressed, Aph2 induces apoptosis. c-Abl and Aph2 have a synergistic effect in apoptosis induction. These results indicate that Aph2 may cooperate with c-Abl in induction of apoptosis.

MATERIALS AND METHODS

**Yeast Two-hybrid Screen**—To produce a bait protein, a type I c-Ab l cDNA fragment (encoding 1091 residues) was subcloned into pSH2–1 to form the LexA DNA binding domain fusion protein. The bait construct was introduced by the standard LAc-polyethylene glycol method into a yeast strain, CTV10–5D, which carried in its genome a β-galactosidase reporter under the regulation of a LexA promoter. The library of cDNAs was derived from the murine myeloid cell line WEHI-3 and was transferred into pGADN OT (20). To screen for c-Ab-interacting proteins, the library was used to transform CTV10–5D carrying the bait and was selected on Leu–His–plates. Interaction was detected by staining for β-galactosidase activity on nitrocellulose replicas. To identify the sequences of Aph2 that interact with c-Abl, different portions of Aph2 cDNA were synthesized by PCR and cloned into pGADN OT and then were transformed into CTV10–5D carrying the bait. β-galactosidase activity was detected on the nitrocellulose replica.

**Northern Analysis**—The tissue distribution of Aph2 was determined by Northern analysis. A blot that contains mRNA from different tissues (CLONTECH) was probed with radio-labeled Aph2. The probe was random primer-labeled full-length Aph2 cDNA.

**Cloning and Expression of Aph2**—To search for c-Abl-interacting proteins, a yeast two-hybrid screen was carried out. The nearly full-length type I c-Abl (aa 1–1091) was used as bait and fused to the DNA binding domain of LexA. The library consists of fusions between the GAL4 activation domain and cDNAs isolated from WEHI-3 myeloid lymphoma. A total of one million yeast colonies were screened, and four were found to carry one previously unidentified gene named Aph2 for abl-philin 2. The gene was also isolated in a separate screen using a C-terminal portion of Ab1 as bait (20). To obtain the full-length cDNA, the 5’-terminal DNA of an initial two-hybrid clone (a 400-bp fragment) was used to screen a mouse liver cDNA library. Clones were isolated, subcloned into pBluescript, and sequenced. The longest cDNA sequence has been deposited to GenBank (accession number AF176814), and the predicted protein sequence is shown in Fig. 1A. The Aph2 gene encodes a 361-amino acid protein.

Aph2 protein shows no strong homology to any known protein in GenBank. A search of the expression data base revealed that Aph2 has a homologue in the human (AAH04535). A search for domains using the domain architecture retrieval tool (DART) revealed two distinct domains, zf-DHHC (aa 157–210) (24) and a 53-amino acid sequence (aa

**RESULTS**

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**Immunoprecipitation and Western Blot**—Transfected cells were washed with cold PBS and lysed in TNEN buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 2 mM EDTA, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml each of pepstatin, leupeptin, and aprotinin). The lysate was clarified by centrifugation at 10,000×g for 10 min at 4°C. Aph2 was immunoprecipitated with monoclonal anti-myc antibody 9E10 (Santa Cruz Biotechnology) and recovered by protein G-agarose (Clontech), which carried in its genome a homologue to creatine kinase isozyme. Aph2 was precipitated with polyclonal antibodies (K-12) (Sambrook et al., 1989) and was precipitated with polyclonal antibodies (K-12) (Sambrook et al., 1989) and then recovered by protein A-agarose. The precipitate was washed in TNEN buffer. c-Abl was precipitated with polyclonal antibodies (K-12) (San-

**Immunohistochemistry**—COS or NIH3T3 cells growing on coverslips were transfected with lipofectAMINE. After 24 h, the cells were per-

**TUNEL Assay**—COS-7 or NIH3T3 cells were transfected with vector DNA expressing Aph2 by the lipofectAMINE method. Cells cultured for 24 h after transfection were fixed in 10% formalin and then assayed for apoptosis using a kit from Oncor, which uses horseradish peroxidase-conjugated antibodies. Counter-staining was performed with Methyl Green.

**Quantitation of Apoptotic Cells**—COS-7 or NIH3T3 cells were transfected with pCR3.1cmv-galactosidase (23) and plasmids expressing c-Abl, Aph2, or both and were cultured for 24 h. Plates were washed with PBS and fixed in a phosphate buffer containing formaldehyde and paraiodohydroxylase for 5 min at room temperature. In each transfection, empty vectors (PSR0 or pMT21) were used to make up the same amount of DNA. After being washed in phosphate buffer containing Nonidet P-40, cells were stained with X-gal at 37°C. When the blue color developed, plates were washed and kept in PBS. β-galactosidase-positive cells were counted at 40 × 10 magnification for five fields of view that represented the approximating cells. For NIH3T3, surviving cells were counted from seven fields of view at the magnification of 10 × 10 because of lower transfection efficiency.

**RESULTS**

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expression of Aph2 in different tissues. B, Western blot showing Aph2 and MBP-Aph2 expressed in COS cells. DNAs expressing wild type and mutant versions of Aph2 were used to transfect COS cells with lipofectAMINE, and cells were lysed after 36 h. Cell lysates were run on SDS-PAGE gel, and Aph2 and mutants were detected with an anti-Myc antibody. C, Western blot showing the expression of mutant Aph2 proteins.

205–257) homologous to the consensus sequence of ATP:guanido phosphotransferase, represented by creatine kinase. The zf-DHHC domain is distinct from the Zinc-finger, LIM, and Ring finger domains and is found in many proteins including Akr1p and Erf2p, both of which are involved in signaling pathways in Saccharomyces cerevisiae (25–28). Fig. 1B shows the alignment of five such proteins. Creatine kinase is the major enzyme in vertebrates that converts creatine to phosphocreatine using ATP as phosphate donor. Phosphocreatine is responsible for transporting energy from the site of ATP synthesis and is also used as an energy buffer (29).

Northern analysis was carried out to determine the expression pattern of Aph2 in mouse tissues. Fig. 2A shows that Aph2 has two transcripts, a major one that is −2 kb in size and a minor one of −3.5 kb. The 2-kb mRNA probably corresponds to the cDNA of Aph2, whereas the 3.5-kb mRNA may represent an alternatively spliced form. Aph2 is expressed in all tissues examined, with a much weaker expression in the spleen. The ubiquitous commercial blot has an even loading of RNA from different tissues examined, with a much weaker expression in the spleen. The ubiquitous commercial blot has an even loading of RNA from different tissues as judged by probing with actin (20). The ubiquitous commercial blot has an even loading of RNA from different tissues as judged by probing with actin (20). The ubiquitous commercial blot has an even loading of RNA from different tissues as judged by probing with actin (20). The ubiquitous commercial blot has an even loading of RNA from different tissues as judged by probing with actin (20).

To determine which domain of the protein interacts with c-Abl, two hybrid assays were carried out (Fig. 3A). We found that any deletion of Aph2 dramatically reduced its affinity for c-Abl. Several fragments truncated at the C terminus displayed quite strong interactions as long as the zf-DHHC domain was retained, whereas all deletions of the N terminus, even in the presence of the zf-DHHC domain, completely abolished the interaction. These results suggest that the N-terminal portion of the protein is important for interaction with c-Abl, although no obvious protein-protein interaction motif is apparent. Similarly, different portions of c-Abl were fused to the DNA binding domain of GAL4 to identify the domains important for the interaction (Fig. 3B). We used the full-length Aph2 fused to the LexA activation domain. These assays suggested that the C-terminal portion of c-Abl interacts with Aph2.

To confirm the interaction in mammalian cells, type IV c-Abl and Aph2 were co-expressed in COS-7 cells, and co-immunoprecipitation assays were performed. First, Aph2-Myc and associated proteins were immunoprecipitated against the c-Myc tag, fractionated on an 8% SDS-PAGE gel, and transferred to nitrocellulose membrane. The blots were probed with antibodies against c-Abl. As shown in Fig. 3C, c-Abl was brought down with Aph2, although pre-immune serum did not precipitate c-Abl. In cells that only express c-Abl anti-Myc antibody did not precipitate c-Abl, confirming the interaction between c-Abl and Aph2 (data not shown). Reciprocally, c-Abl and associated proteins were precipitated with anti-c-Abl antibodies, separated on an 8% SDS-PAGE gel, and transferred to nitrocellulose membrane. The blots were probed with anti-Myc antibody (Fig. 3D). Aph2 was brought down by c-Abl, whereas the pre-immune serum did not bring down Aph2. In cells that only express Aph2, anti-c-Abl antibodies did not precipitate Aph2 (data not shown). These data suggest that there is an interaction between c-Abl and Aph2 in mammalian cells.

Aph2 is not an efficient substrate for c-Abl. In COS-7 cells co-expressing Aph2 and c-Abl, Aph2 was immunoprecipitated using anti-Myc antibody, and tyrosine phosphorylation was determined by Western blot using antibodies that specifically recognize phosphotyrosine. We could not detect any phosphorylated Aph2, although c-Abl autophosphorylation and Abi-1 phosphorylation could be detected easily using the same setting (data not shown).

Localization of Aph2—To study the function of Aph2, the cellular localization of the protein was determined by indirect fluorescent immunocytochemistry using the Myc-tagged Aph2.

![](image1)

FIG. 2. Expression of Aph2. A, Northern blot analysis showing the expression of Aph2 in different tissues. B, Western blot showing Aph2 and MBP-Aph2 expressed in COS cells. DNAs expressing wild type and mutant versions of Aph2 were used to transfect COS cells with lipofectAMINE, and cells were lysed after 36 h. Cell lysates were run on SDS-PAGE gel, and Aph2 and mutants were detected with an anti-Myc antibody. C, Western blot showing the expression of mutant Aph2 proteins.
In COS-7 cells Aph2 was detected exclusively in the cytoplasm, especially in the perinuclear region, which is indicative of endoplasmic reticulum (Fig. 4, A and B). To confirm that Aph2 is localized in the ER, an ER-specific dye (DiOC6) was used. As shown in Fig. 4, C–E, strong co-staining exists between Aph2 and ER. We found no obvious ER retention signal in the Aph2 protein. We next tested for the co-localization of Aph2 and c-Abl. Fig. 4, F–H shows that c-Abl and Aph2 are co-localized, although c-Abl is also localized near the cell surface and in the nucleus of some cells. Our results are consistent with a recent report suggesting that Abl resides on ER and that upon ER stress Abl may translocate to mitochondria and induce apoptosis (30). The localization of Aph2 to the ER suggests that it may play a role related to the ER function of c-Abl.

Using various deletions, we found that the C-terminal portion of Aph2 (aa 226–361) expressed separately was dispersed throughout the cytoplasm, in contrast to the strong perinuclear staining of other fragments of Aph2 (aa 1–226, 150–361, and deletion of the zf-DHHC region) (Fig. 5). These data suggest that more than one signal for ER location may exist, with one residing in the N-terminal portion and another in the zf-DHHC region.

**Ectopic Expression of Aph2 Leads to Apoptosis**

Immuno-staining for Aph2 in both COS-7 (Fig. 4, A and B) and NIH3T3 (data not shown) revealed an interesting aspect of Aph2 function. Both cell types expressing Aph2 were round and smaller, with the nuclei condensed. Some of the cells show nuclei with abnormal shapes, suggesting that these cells are undergoing apoptosis (Fig. 4B). In these cells, Aph2 is organized in a unique structure that is exclusive of the nucleus. In some cells, nuclei were split into two by Aph2. The cells transfected with vector alone did not show this phenotype. To confirm that these cells were apoptotic, TUNEL assays were performed. As shown in Fig. 6, many Aph2 transfected cells were stained positive for apoptosis.

**Aph2 and c-Abl Work Synergistically in the Induction of Apoptosis**

Two-hybrid analysis allowed us to identify a c-Abl-interacting protein. The results of the two-hybrid system are shown in Fig. 3. Interaction between aph2 and c-Abl. Deletion analysis of Aph2 using two-hybrid interaction. A and B, plasmids expressing different mutant forms of Aph2 were introduced into CTY10–5D that was already expressing a LexA-Abl fusion. β-galactosidase activity was measured as an indicator for interaction. ++, dark blue after 1 h at 30 °C; ++, light blue after 4 h; --, no blue after 24 h. C, c-Abl can be co-immunoprecipitated with Aph2. COS cells co-expressing c-Abl and Aph2 were harvested 36 h after the start of transfection and were lysed in TNEN buffer. Aph2 was precipitated with anti-Myc antibody, and c-Abl was detected with anti-c-Abl antibody K-12. D, Aph2 co-immunoprecipitated with c-Abl. COS cells co-expressing c-Abl and Aph2 were lysed, c-Abl was precipitated with K-12, and Aph2 was detected with anti-Myc antibody 9E10. IP, immunoprecipitate. NLS, nuclear localization signal; PTK, protein tyrosine kinase.
struct that expresses β-galactosidase. If the expression of the gene of interest causes apoptosis, the number of β-galactosidase-positive cells will be reduced. This method has been used successfully to study the effect of c-Abl on apoptosis and in other cases (10). In our experiments, we used both COS-7 and NIH3T3 cells. The data presented below are from COS-7 cells because of its high transfection efficiency, but similar results were obtained from these two cell lines.
expression level was reduced dramatically by the expression of Aph2 (Fig. 7). Similarly, when cells were transfected with a fixed amount of Aph2 DNA, increasing the amount of c-Abl DNA dramatically reduced the level of Aph2 expression (data not shown). On the other hand, Abi-1, another c-Abl-interacting protein, did not down-regulate c-Abl expression when co-expressed (data not shown). These results suggest either that cells co-expressing Aph2 and c-Abl have a higher tendency to undergo apoptosis, that Aph2 and c-Abl proteins are mutually down-regulated, or both. The results shown in Fig. 6 favor the conclusion that down-regulation is attributable to a loss from the cultures of those cells expressing the two proteins, resulting from the synergistic effects of c-Abl and Aph2 on apoptosis induction. Aph2 is under the control of a hybrid SV40/HTLV promoter, and c-Abl is under the control of the adenovirus major late promoter. The down-regulation could not be explained by competition for transcriptional machinery. The C portion of Aph2, under the control of the same promoter, did not have a similar effect. The N portion of Aph2 did down-regulate the expression of c-Abl (data not shown). These results on apoptosis induction (Fig. 6C), and down-regulation of c-Abl obtained with truncated forms of Aph2 also support our conclusion that the down-regulation is attributable to a loss from the cultures of those cells expressing both c-Abl and Aph2.

**DISCUSSION**

We have cloned and characterized a novel gene, aph2, encoding a c-Abl-interacting protein. This protein has a cysteine-rich motif (zf-DHHC) that is found in many proteins from yeast to human. Most of these protein sequences are predicted from genomic DNA sequences and therefore are not well characterized. Other cysteine-rich motifs include the better-known Zinc finger, Ring finger, and LIM domains. Although Zinc finger domains often are involved in protein-DNA interaction, both Ring finger and LIM are involved in protein-protein interactions. In addition, Ring finger domains also play an important role in protein ubiquitination and degradation. The zf-DHHC motif of Aph2 probably does not bind DNA because Aph2 was localized in the ER. Two yeast proteins (Akr1p and Erf2p) containing the motif were not localized in the nucleus either. The motif may be involved in disulfide bond formation inter- or intra-molecularly. Aph2 expressed in either bacteria or mammalian cells is prone to aggregation and runs as a >200 Kp band on SDS-PAGE gels. Deletion of the domain facilitated the solubilization of Aph2.

The functions of zf-DHHC-bearing proteins may be diverse. Two such proteins from yeast have been characterized to date, Akr1p and Erf2p. Akr1p interacts physically with the pheromone receptor-coupled G protein, and a genetic interaction has been observed with cdc42p and also with an ARF GAP, Gcs1p(25-27, 32). Erf2p is required for the proper localization and palmitoylation of Ras proteins in yeast (28). These proteins do not suggest any simple role for the motif. Another clue regarding zf-DHHC function is that in some proteins it coexists with ankyrin repeats, which are important for protein-protein interactions, e.g., Akr1p and an uncharacterized protein (NP_014667) of S. cerevisiae, a hypothetical protein (T338570) of Caenorhabditis elegans, a hypothetical protein (AAF48554) of Drosophila melanogaster, and an uncharacterized protein (NP_061901) of human. Whether a functional connection exists between zf-DHHC and ankyrin repeats is unclear. Two more mammalian proteins containing zf-DHHC also have been deposited into GenBank™. One is named as rec (reduced expression in cancer, accession number NP_057437), and the other is a protein from the human pancreatic library (C160RF1). The function of either rec or C160RF1 is not known.

Aph2 has a 53-amino acid stretch sharing homology with...
ATP-guanido phosphotransferase consensus sequence found in creatine and arginine kinases. The homology resides in domain I (N-terminal) of the creatine kinase family, comprising α-helices 4 and 5, which may be involved in substrate interaction. Domain II is important for ATP binding and enzymatic activity (29). Because Aph2 has homology to only 53 amino acids of the 380-amino acid consensus sequence, it is unlikely that Aph2 plays a role in the energy buffer or transport system.

How does overexpression of Aph2 induce apoptosis? Based on the localization of Aph2 and the fact that cyclosporin A could not protect cells from apoptosis induced by Aph2 (data not shown), it is likely that Aph2 induces apoptosis in an ER stress-related pathway. Accumulation of misfolded proteins or an alteration in calcium homeostasis causes ER stress, which leads to apoptosis of cells if the damages are not fixed. Caspase 12 is localized in the ER and is responsible for ER stress-induced apoptosis. Bcl2, an anti-apoptotic protein, also was found to be located in the ER in addition to mitochondria. Depletion of ER calcium by the overexpression of Bcl2 plays an important anti-apoptotic role (33). Overexpression of Aph2 somehow may stress the ER and subsequently induce apoptosis. Whether Aph2 affects protein folding or calcium homeostasis needs to be studied. Aph2 has been implicated to play a role in the energy buffer or transport system. Somehow may stress the ER and subsequently induce apoptosis.

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REFERENCES
1. Wang, J. Y. (2000) Oncogene 19, 5643–5650
2. Van Etten, R. A. (1999) Trends Cell Biol. 9, 179–186
3. Lewis, J. M., Baskaran, R. F., Taagepera, S., Schwartz, M. A., and Wang, J. Y. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 15174–15179
4. Taagepera, S., McDonald, D. F., Loeb, J. E., Whitaker, L. L., McElroy, A. K., Wang, J. Y., and Hope, T. J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7457–7462
5. Schwarzberg, P. L., Stall, A. M., Hardin, J. D., Bowdich, K. S., Humar, T. F., Boast, S., Harbison, M. L., Robertson, E. J., and Goff, S. P. (1991) Cell 65, 1165–1175
6. Tybulewicz, V. L., Crawford, C. E., Jackson, P. K., Bronson, R. T., and Mulligan, R. C. (1991) Cell 65, 1153–1163
7. Li, B., Boast, S., de los Santos, K., Schierene, I., Quiriz, M., Teitelbaum, S. L., Tondruzi, M. M., and Goff, S. P. (2000) Nat. Genet. 24, 304–308
8. Krush, G. D., Pereg, R., Miki, T., and Aaronson, S. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5802–5806
9. Koleske, A. J., Gifford, A. M., Scott, M. L., Nee, M., Bronson, R. T., Mizek, K. A., and Baltimore, D. (1998) Neuron 21, 1259–1272
10. Theis, S., Roemer, K. (1998) Oncogene 17, 557–564
11. Cong, F., and Goff, S. P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13819–13824
12. Huang, Y., Yuan, Z. M., Ishiko, T., Nakada S., Utsugisawa, T., Kato, T., Kharbanda, S., and Kufe, D. W. (1997) Oncogene 15, 1847–1952
13. Yuan, Z. M., Huang, Y., Ishiko, T., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1437–1440
14. Gong, J. G., Costanzo, A., Yang, H. Q., Melino, G., Kaelin, W. G., Jr., Levrero, M., and Wang, J. Y. (1999) Nature 399, 806–809
15. Dorsch, M., and Goff, S. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13313–13316
16. Vigneri, P., and Wang, J. Y. (2001) Nat. Med. 7, 228–234
17. Raitano, A. B., Whang, Y. E., and Sawyers, C. L. (1997) Biochem. Biophys. Acta 1333, F201–F216
18. Wen, S. T., and Van Etten, R. A. (1997) Genes Dev. 11, 2456–2467
19. Agami, R., and Shaul, Y. (1998) Oncogene 16, 1779–1788
20. Shi, Y., Alin, K., and Goff, S. P. (1996) Genes Dev. 9, 2583–2597
21. Takebe, Y., Seiki, M., Fujisawa, J., Hiy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988) Mol. Cell. Biol. 8, 466–472
22. An, D. S., Koyanagi, Y., Zhao, J. Q., Akkina, R., Bristol, G., Yamamoto, N., Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988) Mol. Cell. Biol. 8, 466–472
23. Liu, Z. G., Hsu, H., Goeddel, D. V., and Karin, M. (1996) Cell 87, 565–576
24. Puttilina, T., Wong, P., and Gentleman, S. (1999) Mol. Cell. Biochem. 193, 219–226
25. Givan, S. A., and Sprague, G., Jr. (1997) Mol. Biol. Cell 8, 1317–1327
26. Kao, L. R., Peterson, J. R., Bender, L., and Bender, A. (1996) Mol. Cell. Biol. 16, 168–178
27. Pyriani, P. M., and Hartwell, L. H. (1996) Mol. Cell. Biol. 16, 2614–2626
28. Bartels, D. J., Mitchell, D., Deng, X., and Deschenes, R. J. (1999) Mol. Cell. Biol. 19, 6775–6787
29. Wyse, M., and Shorttahc-Dunou, R. (2000) Physiol. Rev. 80, 1107–1213
30. Io, X., Pandey, P., Mishra, N., Kumar S., Narula, N., Kharbanda, S., Saxena, S., and Kufe, D. (2001) Mol. Cell. Biol. 21, 6233–6242
31. Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999) Nature 399, 809–813
32. Feng, Y., and Davis, N. G. (2000) Mol. Cell. Biol. 20, 5350–5359
33. Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F., Pozzan, T., and Rizzuto, R. (2001) EMBO J. 20, 2690–2701