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The phosphoinositide 3-kinase/Akt pathway is activated by daunorubicin in human acute myeloid leukemia cell lines

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Abstract Daunorubicin induces apoptosis in myeloid leukemia cells by activation of neutral sphingomyelinase and ceramide generation occurring 4–10 min after daunorubicin addition. We show here that daunorubicin is able to increase the phosphoinositide 3-kinase activity and enhance intracellular phosphoinositide 3-kinase lipid products prior to ceramide generation. Daunorubicin activates Akt, a downstream phosphoinositide 3-kinase effector. Interestingly, the phosphoinositide 3-kinase inhibitors wortmannin and LY294002 accelerate daunorubicin-induced apoptosis in U937 cells. The phosphoinositide 3-kinase/Akt pathway has been involved in cell survival following serum deprivation, tumor necrosis factor α, anti-Fas and UV radiations. Our results suggest that anti-tumor agents such as daunorubicin may also activate anti-apoptotic signals that could contribute to drug resistance.

Key words: Apoptosis; Phosphoinositide 3-kinase; Akt; Daunorubicin

1. Introduction

Daunorubicin (DNR), an anthracycline widely used in the treatment of acute myeloid leukemia (AML), induces apoptosis in some but not all myeloid leukemic cells. For example, DNR at 0.5–1 μM induces internucleosomal DNA fragmentation and morphological changes characteristic of apoptosis in U937 and HL-60 cells [1]. The nature of the signaling pathways which initiate DNR-triggered apoptosis is still debated. We have reported that at these concentrations, DNR stimulates sphingomyelin (SM) hydrolysis and subsequent ceramide generation due to neutral sphingomyelinase (SMase) activation [2]. The role of ceramide released from SM hydrolysis in DNR-induced apoptosis was supported by further studies indicating that SMase inhibition results not only in the inhibition of ceramide generation but also in the inhibition of apoptosis in DNR-treated cells [3,4]. The SM cycle is a common apoptotic signaling pathway shared by many other cytotoxic agents including vincristine, tumor necrosis factor α (TNFα), CD95 agonists and radiations [5].

These studies and others emphasize the role of ceramide as a key mediator in DNR-induced apoptosis. However, ceramide-induced apoptosis is controlled by several factors including Bcl-2 expression [6] and protein kinase C (PKC) activity [7]. Therefore, external or intracellular stimuli which result in Bcl-2 overexpression and/or PKC stimulation may limit the DNR-induced apoptosis and cytotoxicity. Previous studies have reported that doxorubicin activates PKC in various cell systems [8] and stimulates phospholipase C and subsequent diacylglycerol (DAG) production in melanoma cells [9]. These observations raise the intriguing possibility that anthracyclines interfere with several membrane-associated enzyme activities which initiate either cell death or survival signals and that the balance between these two opposite pathways may influence the cellular response.

Among other enzymes which are involved in signal transduction pathways, phosphoinositide 3-kinase (PI3K) plays an important role. PI3Ks are a family of enzymes that catalyze the phosphorylation of inositol lipids at the D3 position of the inositol ring, generating new intracellular second messengers [10]. The lipid products of PI3K are phosphatidylinositol-3-phosphate (PtdIns-3-P), phosphatidylinositol-3,4-bisphosphate (PtdIns-3,4-P_2) and phosphatidylinositol-3,4,5-trisphosphate (PtdIns-3,4,5-P_3). In vivo, PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 have been demonstrated to interact with downstream effectors. They act directly or indirectly on serine/threonine kinase Akt which has been involved in the regulation of cell survival (for a review, see [11]). In addition, PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 stimulate calcium-insensitive novel PKC δ, ε, η isoforms [12] as well as the atypical PKC ζ isoform [13]. The use of PI3K inhibitors, wortmannin (WM) and LY294002 and PI3K mutants has allowed us to demonstrate a role for PI3K in the cell survival after various stresses including serum deprivation [14,15], ionizing radiation [16], UV irradiation [17], TNFα [18] and CD-95 [19].

We hypothesized that the stress induced by DNR could

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Abbreviations: AML, acute myeloid leukemia; DAG, diacylglycerol; DNR, daunorubicin; FCS, fetal calf serum; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; HPLC, high performance liquid chromatography; IRS-1, insulin receptor substrate-1; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PtdIns-3-P, phosphatidylinositol-3-phosphate; PtdIns-3,4-P_2, phosphatidylinositol-3,4-bisphosphate; PtdIns-4,5-P_2, phosphatidylinositol-4,5-bisphosphate; PtdIns-3,4,5-P_3, phosphatidylinositol-3,4,5-trisphosphate; ROS, radical oxygen species; SM, sphingomyeline; SMase, sphingomyelinase; TLC, thin layer chromatography; TNF, tumor necrosis factor; WM, wortmannin

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stimulate PI3K activity that might, in turn, influence drug-induced apoptosis.

2. Materials and methods

2.1. Chemicals and antibodies

DNR was purchased from Roger Bellon (Neuilly/Seine, France). LY294002 was provided by Calbiochem (Meudon, France). All other reagents were obtained from Sigma (St-Quentin Fallavier, France). Polyclonal antibody anti-p85 (06-195) was purchased from UBI (Euromedex, Souffelweyersheim, France). Polyclonal Akt (phosphorylation state-independent) and phospho-specific Akt (Ser-473) were provided by New England Biolabs (Ozyme, Montigny le Bretonneux, France). [γ-^{32}P]ATP (7000 Ci/mmol) and [^{32}P]orthophosphate were obtained from ICN (Orsay, France).

2.2. Leukemic cell lines

The monocytic U937 cell line was obtained from the ATCC (Rockville, MD, USA) and was cultured in RPMI supplemented with 10% fetal calf serum (FCS), 2 mM glutamine and penicillin/streptomycin.

2.3. In vitro lipid kinase assay

In vitro lipid kinase was assayed as previously described [20]. Briefly, exponentially growing cells were incubated in RPMI containing 1% FCS overnight. Cells were then incubated in serum-free RPMI with or without WM (100 nM) for 30 min. After various times of incubation with DNR, PI3K was immunoprecipitated prior to the assay. After washing, the pellets were resuspended in 30 μl buffer 1 (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 0.4 mM EDTA and 0.4 mM Na_2HPO_4). 10 μl of 1 mg/ml phosphatidylinositol previously sonicated and 10 μl reaction buffer (10 mM $MgCl_2$, 50 μM ATP containing 0.3 μCi/sample of [γ-^{32}P]ATP in buffer 1) were incubated with the enzyme for 10 min at room temperature. The reaction was stopped by the addition of 15 μl of 4 N HCl. Lipids were extracted with methanol/chloroform (1:1, v/v). Lipids in the organic phase were separated by thin layer chromatography (TLC). After autoradiography, the PtdIns-3-P fractions were scraped off and the incorporated radioactivity was assayed by scintillation counting.

2.4. Intracellular PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 assay

Exponentially growing cells were incubated overnight in RPMI containing 1% FCS. They were incubated in serum- and phosphate-free RPMI for 4 h with 0.5 Ci/mmol [^{32}P]orthophosphate at 37°C, then washed with serum- and phosphate-free buffer before the addition of WM (100 nM for 30 min) and/or DNR (1 μM for different times). The reaction was stopped by the addition of 2.4 N HCl at 4°C. Lipids were extracted and analyzed as previously described [21]. For PtdIns-3,4,5-P_3 level measurements, lipids were separated by TLC and radiolabelled PtdIns-3,4,5-P_3 was scraped off, separated and analyzed by high performance liquid chromatography (HPLC) on a Partisphere-SAX anion exchange column [22].

2.5. Akt activation

U937 cells were treated as for the lipid kinase activity. After various times of incubation, cells were lysed and total cell proteins were separated in a 7.5% SDS-polyacrylamide gel and transferred on nitrocellulose. Activated Akt (phosphorylated on Ser-473) was detected using a phospho-specific Akt antibody and normalized to the total Akt after probing with an Akt antibody independent of the phosphorylation status [23].

2.6. DNA ladders

Exponentially growing cells were pre-incubated overnight in RPMI containing 1% FCS, then pre-treated or not with WM (50 and 100 nM) or LY294002 (10 and 25 μM) in serum-free RPMI for 30 min. DNR at 0.2 or 1 μM was added to the cells for 1 h. After washing, cells were incubated in DNR-free medium in the presence or absence of PI3K inhibitors for 3 or 5 h. After cell centrifugation at 20 000 × *g*, the pellets were incubated in lysis buffer (10 mM Tris-HCl, 10 mM EDTA, 10 mM NaCl, 0.5% SDS and 0.5 mg/ml proteinase K) for 1 h at 50°C, then with RNAse A for 2 h at 50°C. After precipitation, DNA was electrophoresed in a 1.8% agarose gel in TBE at 50 V. The photographs of gels stained with ethidium bromide were recorded.

3. Results

3.1. DNR activates p85/p110 PI3K in U937 cells

To determine whether PI3K could play a role in cell fate after DNR treatment, we first investigated whether DNR could stimulate a PI3K activity in U937 cells. PI3K activation was determined by assaying the in vitro lipid kinase activity in p85 immunoprecipitates. The results showed a rapid but moderate (2-fold) increase in the PI3K activity induced by 1 μM DNR (Fig. 1A). At 10 min, the PI3K activity returned to the basal level. Similar experiments were conducted in the presence of the PI3K inhibitor WM. A pretreatment with 100 nM WM for 30 min inhibited the basal and DNR-induced PI3K activity by 80–90% (Fig. 1B).

We examined the dose-response effect of DNR on the PI3K activity. At a low DNR concentration (0.1 μM), a moderate PI3K activation started 5 min after DNR treatment and was sustained at 10 min, while it declined back to the basal level at 30 min (Fig. 1C). A second peak of activation occurred between 1 and 2 h. At 3 h, PI3K drastically decreased and returned to the basal level (data not shown). At DNR concentrations comprised between 0.2 and 1 μM, the PI3K activ-

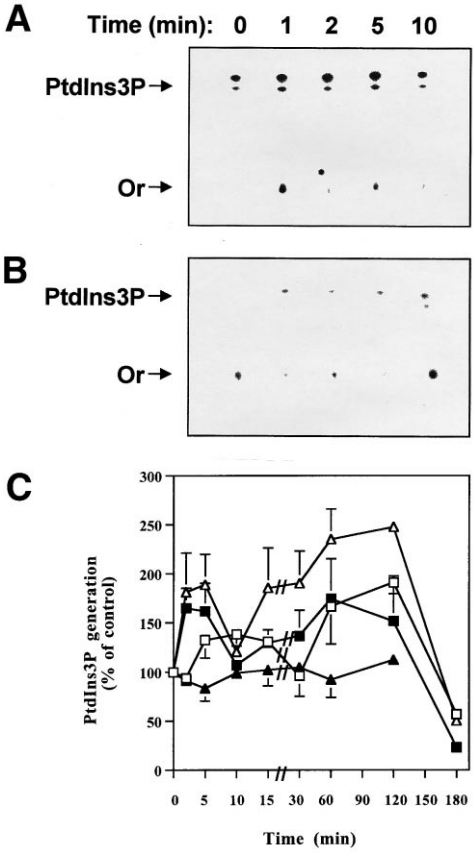

Fig. 1. Time-course and dose-response of lipid kinase activation by DNR. (A) U937 cells were treated with 1 μM DNR. Cells were lysed at the indicated time and the lysates were immunoprecipitated with anti-p85 antibody. Following reaction with PtdIns and [γ-^{32}P]ATP, the phospholipids were separated by TLC, before autoradiography (Or: origin). (B) U937 cells were pre-incubated with 100 nM WM for 30 min, before DNR addition. (C) U937 cells were treated with DNR at 0.1 μM (open square), 0.2 μM (open triangle), 1 μM (closed square), 2 μM (closed triangle). Data in A and B are from one experiment representative of 4–6 experiments. Data in C are the mean ± S.D. of 4–6 independent experiments.

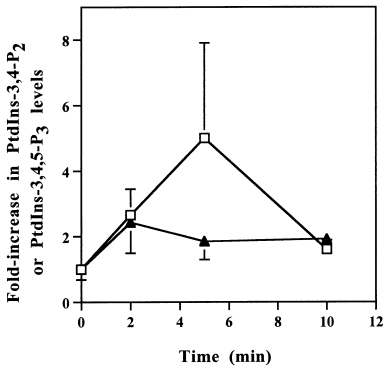

Fig. 2. DNR-induced generation of PI3K lipid products in U937 cells. After labelling with [^{32}P]orthophosphate for 4 h, cells were treated with 1 μM DNR. At the indicated time, cells were lysed and lipids were extracted. PtdIns-3,4-P_2 (open square) and PtdIns-3,4,5-P_3 (closed triangle) levels were determined after HPLC and TLC prior to HPLC separation, respectively. The data are means ± S.D. from three and five independent experiments, respectively.

ity was stimulated earlier (2 min). The 1.6–2-fold increase occurred between 2 and 5 min and then declined at 10 min. A second peak started at 15 min. A high level of PI3K activity was maintained for 2 h, then, the PI3K activity was drastically reduced (Fig. 1C) and remained low (data not shown). Higher DNR concentrations were not able to significantly stimulate PI3K activity, suggesting a dose-dependent effect of DNR such as previously observed on ceramide generation [2].

3.2. DNR induces the intracellular accumulation of PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3

To determine whether the lipid products resulting from PI3K activity were increased in DNR-treated cells, we measured the levels of these lipids in U937 cells treated by 1 μM DNR after separation by HPLC. The addition of DNR to U937 cells resulted in a transient accumulation of PtdIns-3,4,5-P_3 and PtdIns-3,4-P_2 (Fig. 2). The increase in PtdIns-3,4-P_2 levels was higher but the kinetics of PtdIns-3,4-P_2 accumulation were slower than that of PtdIns-3,4,5-P_3. The results are expressed relative to basal levels of PtdIns-3,4-P_2 (1003 ± 182 cpm) and PtdIns-3,4,5-P_3 (1384 ± 449 cpm). Compared with the control status, the PtdIns-3,4-P_2/PtdIns-4,5-P_2 ratio was increased by a factor of two and six at 2 and 5 min, respectively, then declined back to the basal level at 10 min (data not shown). Pretreatment with 100 nM WM decreased the basal levels of PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 by 80 and 70%, respectively (data not shown).

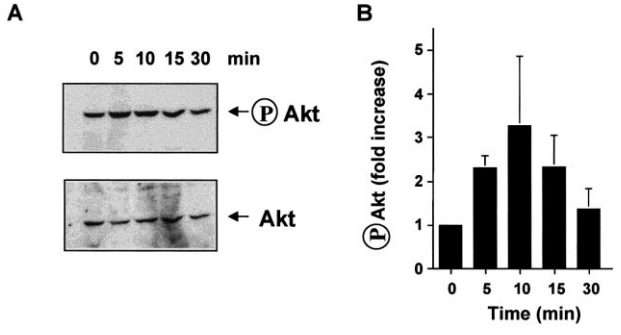

Fig. 3. DNR-induced activation of Akt. (A) U937 cells were treated with 1 μM DNR. Cells were lysed and proteins were separated on 7.5% SDS-PAGE, transferred to nitrocellulose and immunoblotted with Akt and phospho-specific Akt antibodies. (B) The phosphorylated Akt bands were quantified by a phosphorimager Storm-System analysis using Imagequant software and normalized to the amounts of total Akt. The data shown in B are means ± S.D. of three independent experiments.

3.3. Akt is activated by DNR addition to U937 cells

Akt has been described as a downstream effector of PI3K in a number of models that involve activation of PI3K in survival signaling. We investigated whether Akt could be activated in U937 cells in response to DNR. The activation of Akt is dependent on phosphorylation at two sites, Ser-473 and Thr-308. We measured the extent of Akt activation using an antibody specific of phosphorylated Akt at Ser-473 [24]. DNR at 0.2 or 1 μM increased the levels of the phosphorylated Akt form at 5 min (Fig. 3A and B). The levels of phosphorylated Akt expression declined back to the basal level at 15–30 min and did not increase at longer times (data not shown). Pretreatment with 100 nM WM or 25 μM LY294002 inhibited the expression of the Akt activated form following DNR treatment by 90–95% (data not shown). These results show that Akt is a downstream effector of the DNR-induced activation of PI3K.

3.4. Inhibition of PI3K accelerates cell death by apoptosis

We tested whether PI3K inhibitors could alter DNR-triggered apoptosis in human myeloid cells. Apoptosis characterized by DNA ladders has been described in U937 cells 6 h after 1 μM DNR treatment [1]. However, WM pretreatment accelerated DNA fragmentation. At 4 h, DNA ladders were observed in DNR-treated U937 cells pre-treated with 100 nM

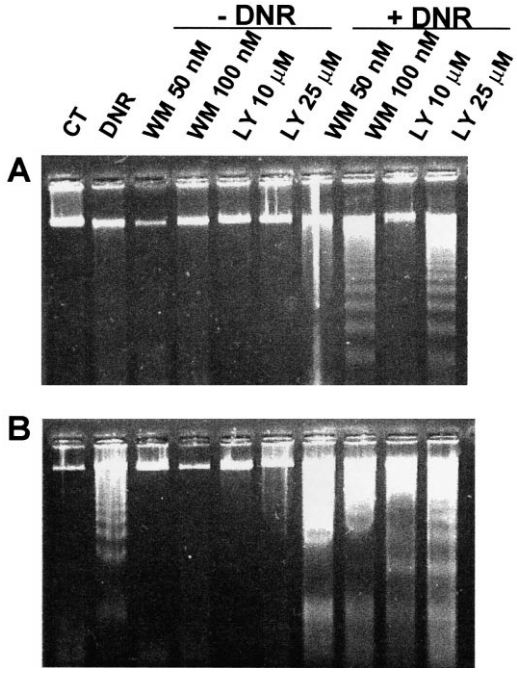

Fig. 4. Effects of WM and LY294002 on DNR-induced apoptosis in U937 cells. Internucleosomal DNA fragmentation was determined in U937 cells pre-incubated or not with WM (50 or 100 nM) or LY294002 (LY) (10 or 25 μM), then treated with 1 μM DNR and further incubated with DNR-free medium with or without PI3K inhibitors (A) for 4 h, (B) for 6 h. The data in A and B are from one experiment representative of three independent experiments.

WM or 25 μM LY294002, while no ladder was observed after cell treatment with DNR or inhibitors alone (Fig. 4A). At 6 h, PI3K inhibitors alone did not induce DNA fragmentation but DNA ladders were observed in DNR-treated cells and amplified in WM- or LY294002-pre-treated cells even at lower inhibitor concentrations (Fig. 4B).

4. Discussion

Our study shows that DNR enhanced the in vitro lipid kinase activity as well as the generation of lipid products of PI3K in U937 cells. PtdIns-3,4,5-P_3 synthesis was rapid and transient and PtdIns-3,4-P_2 accumulation was slightly delayed. Although PI3K activation and generation of its lipid products were moderate compared to those observed for example following growth factor addition, this increase was reproducibly observed. The first peak of lipid kinase activation was transient and concomitant with the appearance of lipid products. A 2-fold stimulation of the in vitro lipid kinase activity was also observed 2–5 min after DNR addition in the HL-60 myeloid cell line, indicating that PI3K activation is not restricted to the U937 cell model (data not shown). A second activation was maintained for about 2 h and had no effect on Akt activation. Interestingly, the PI3K activity drastically decreased at 3 h, prior to DNA fragmentation and PARP cleavage. Except at 0.1 μM, this was not reversible. The mechanisms by which DNR stimulates PI3K are unclear. Whether DNR interferes with the tyrosine phosphorylation status of receptor tyrosine kinases, intracellular substrates such as insulin receptor substrate-1 (IRS-1) or Src family proteins, as previously shown for other stimuli, has not been investigated yet. However, radical oxygen species (ROS) may interfere with the function of proteins known to interact with PI3K. Exposure to H_2O_2 results in tyrosine phosphorylation of IRS-1 and its specific association with the p85 adaptor of PI3K activates p21Ras [24] which may interact directly with the p110-PI3K [25]. H_2O_2 also induces tyrosine phosphorylation of pp60c-src and PDGFβ receptor [26]. Therefore, the oxidative stress generated by DNR [27] may play a role in PI3K activation. Such hypotheses are currently under study in our laboratory.

Our results suggest that the PI3K activation plays a role in DNR-treated leukemic cells. We failed to observe a significant effect of two different PI3K inhibitors on the cell clonogenicity after DNR treatment. However, we showed that PI3K inhibitors facilitated apoptosis in U937 cells. Internucleosomal DNA fragmentation was accelerated by pretreatment with inhibitors in DNR-treated U937 cells. DNR-induced poly(ADP-ribose)polymerase cleavage, another specific event of apoptosis, was also accelerated in the presence of PI3K inhibitors (data not shown). Moreover, PI3K activation was abolished after 3 h, suggesting PI3K inhibition when cells are engaged in apoptosis. These results suggest a protective role for PI3K activation and are in agreement with those previously described with UV-, TNFα- and anti-Fas-induced apoptosis [17,18].

The DNR-induced PI3K activation appeared to be much lower than that observed after transfection of oncogenes [28] or cytokine stimulation [29]. However, one can speculate that PI3K could impair DNR-induced apoptosis and/or cytotoxicity. The fact that BCR/ABL transfection inhibits DNR-induced apoptosis [30] and confers resistance to DNR (and to other compounds) supports the general idea that a sustained PI3K activation may be involved in drug resistance.

Our study showed that DNR activated Akt and that Akt activation was inhibited in a PI3K-dependent manner. This activation occurred in response to the earlier peak of PI3K activation. In response to growth factors, PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 can interact with Akt. This interaction most likely plays a role in the translocation of Akt to the membrane where it can be activated by upstream kinases [31] also regulated by PI3K lipid products [32]. Although the function of Akt is not fully understood, most recent studies suggest a role for Akt in the cell survival [17,33]. The finding that H_2O_2-induced Akt activation is dependent on PI3K [34] supports a role for DNR-induced ROS in the DNR-triggered PI3K/Akt pathway. The mechanisms by which Akt exerts its protective function are still under investigation. It has been proposed that Akt could regulate the Bcl-2 expression [35]. In our model, PI3K inhibitors did not change the Bcl-2 expression following cell exposure to DNR (data not shown), indicating that Bcl-2 expression is not controlled by the PI3K/Akt pathway. Similar conclusions were reported by Hay and colleagues [15,23]. Recently, it has been proposed that Akt phosphorylates and inhibits BAD, a pro-apoptotic member of the Bcl-2 family, by cytosolic sequestration and prevention of binding to Bcl-xL [36]. This hypothesis is under current investigation in our laboratory. Another mechanism involves inhibition of Ced3/ICE-like activity by PI3K and Akt [16] while PI3K inhibition enhanced caspase 3 activation [18]. Whether PI3K could regulate caspase activation during DNR-induced apoptosis in myeloid cells needs to be investigated.

In summary, our study shows that DNR activates the PI3K/Akt pathway and that PI3K inhibitors accelerate DNR-induced apoptosis. The control of PI3K activity could be an attractive way to modulate the cytotoxicity of chemotherapeutic drugs in cancer cells.

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References

[1] Quillet-Mary, A., Mansat, V., Duchayne, E., Come, M.G., Allouche, M., Bailly, J.D., Bordier, C. and Laurent, G. (1996) Leukemia 10, 417–425.
[2] Jaffrézou, J.P., Levade, T., Bettaïeb, A., Andrieu, N., Bezombes, C., Maestre, N., Vermeesch, S., Rousse, A. and Laurent, G. (1996) EMBO J. 15, 2417–2424.
[3] Mansat, V., Bettaïeb, A., Levade, T., Laurent, G. and Jaffrézou, J.P. (1997) FASEB J. 11, 695–702.
[4] Mansat, V., Laurent, G., Levade, T., Bettaïeb, A. and Jaffrézou, J.P. (1997) Cancer Res. 57, 5300–5304.
[5] Hannun, Y.A. (1996) Science 274, 1855–1861.
[6] Allouche, M., Bettaïeb, A., Vindis, C., Rousse, A., Grignon, C. and Laurent, G. (1997) Oncogene 14, 1837–1845.
[7] Jarvis, W.D., Fornari Jr., F.A., Browning, J.L., Gewirtz, D.A., Kolesnick, R.N. and Grant, S. (1994) J. Biol. Chem. 269, 31685–31692.
[8] Ohkawa, K., Hatano, T., Isonishi, S., Takada, K., Joh, K. and Matsuda, M. (1994) Int. J. Oncol. 4, 655–659.
[9] Posada, J., Vichi, P. and Tritton, T.R. (1989) Cancer Res. 49, 3334–3339.
[10] Toker, A. and Cantley, L.C. (1997) Nature 387, 673–676.
[11] Downward, J. (1998) Curr. Opin. Cell Biol. 10, 262–267.

[12] Toker, A., Meyer, M., Reddy, K.K., Falk, J.R., Aneja, R., Aneja, S., Parra, A., Burns, D.J., Ballas, L.M. and Cantley, L.C. (1994) J. Biol. Chem. 269, 33258–33267.
[13] Nakanishi, H., Brewer, K.A. and Exton, J.H. (1993) J. Biol. Chem. 268, 13–16.
[14] Yao, R. and Cooper, G.M. (1995) Science 267, 2003–2006.
[15] Kennedy, S.G., Wagner, A.J., Conzen, S.D., Jordan, J., Bellacosa, A., Tsichlis, P.N. and Hay, N. (1997) Genes Dev. 11, 701–713.
[16] Price, B.D. and Youmell, M.B. (1996) Cancer Res. 56, 246–250.
[17] Kulik, G., Klippel, A. and Weber, M.J. (1997) Mol. Cell. Biol. 17, 1595–1606.
[18] Fujita, E., Kouroku, Y., Miho, Y., Tsukahara, T., Ishiura, S. and Momoi, T. (1998) Cell Death Differ. 5, 289–297.
[19] Hausler, P., Papoff, G., Eramo, A., Reif, K., Cantrell, D.A. and Ruberti, G. (1998) Eur. J. Immunol. 28, 57–69.
[20] Kowalski-Chauvel, A., Pradayrol, L., Vaysse, N. and Seva, C. (1997) Biochem. Biophys. Res. Commun. 236, 687–692.
[21] Guinebault, C., Payrastre, B., Racaud-Sultan, C., Mazarguil, H., Breton, M., Mauco, G., Plantavid, M. and Chap, H. (1995) J. Cell. Biol. 129, 831–842.
[22] Ireton, K., Payrastre, B., Chap, H., Ogawa, W., Sakaue, H., Kasuga, M. and Cossart, P. (1996) Science 274, 780–782.
[23] Eves, E.M., Xiong, W., Bellacosa, A., Kennedy, S.C., Tsichlis, P.N., Rosner, M.R. and Hay, N. (1998) Mol. Cell. Biol. 18, 2143–2152.
[24] Lander, H.M., Ogiste, J.S., Teng, K.K. and Novogrodski, A. (1995) J. Biol. Chem. 270, 21195–21198.
[25] Rodrigez-Viciana, P., Warne, P.H., Vanhaesebroeck, B., Waterfield, M.D. and Downward, J. (1996) EMBO J. 15, 2442–2451.
[26] Gonzalez-Rubio, M., Voit, S., Rodriguez-Puyol, D., Weber, M. and Marx, M. (1996) Kidney Int. 50, 164–173.
[27] Alegria, A.E., Samuni, A., Mitchell, J.B., Riesz, P. and Russo, A. (1989) Biochemistry 28, 8653–8658.
[28] Skorski, T., Kanakaraj, P., Nieborowska-Skorska, M., Ratajczak, M.Z., Wen, S.C., Zon, G., Gewirtz, A.M., Perussia, B. and Calabretta, B. (1995) Blood 86, 726–736.
[29] Sattler, M., Salgia, R., Shrikhande, G., Verma, S., Pisick, E., Prasad, K.V.S. and Griffin, J.D. (1997) J. Biol. Chem. 272, 10248–10253.
[30] Bedi, A., Barber, J.P., Gauri, C., Bedi, G.C., El-Deiry, W.S., Sidransky, D., Vala, M.S., Akhtar, A.J., Hilton, J. and Jones, R.J. (1995) Blood 86, 1148–1158.
[31] Andjelkovic, M., Alessi, D.R., Meier, R., Fernandez, A., Lamb, N.J.C., Frech, M., Cron, P., Cohen, P., Lucocq, J.M. and Hemmings, B.A. (1997) J. Biol. Chem. 272, 31515–31524.
[32] Stephens, L., Anderson, K., Stokoe, D., Erdjument-Bromage, H., Painter, G.F., Holmes, A.B., Gaffney, P.R., Reese, C.B., McCormick, F., Tempst, P., Coadwell, J. and Hawkins, P.T. (1998) Science 279, 710–714.
[33] Dudek, H., Datta, S.R., Franke, T.F., Birnbaum, M.J., Yao, R., Cooper, G.M., Segal, R.A., Kaplan, D.R. and Greenberg, M.E. (1997) Science 275, 661–665.
[34] Shaw, M., Cohen, P. and Alessi, D.R. (1998) Biochem. J. 336, 241–246.
[35] Ahmed, N.N., Grimes, H.L., Bellacosa, A., Chan, T.O. and Tsichlis, P.N. (1997) Proc. Natl. Acad. Sci. USA 94, 3627–3632.
[36] del Peso, L., Gonzales-Garcia, M., Page, C., Herrera, R. and Nunez, G. (1997) Science 278, 687–689.