RESEARCH COMMUNICATION

Sphingolipid metabolites differentially regulate extracellular signal-regulated kinase and stress-activated protein kinase cascades

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The mitogen-activated protein kinase (MAPK) signalling pathway serves to translocate information from activated plasma-membrane receptors to initiate nuclear transcriptional events. This cascade has recently been subdivided into two analogous pathways: the extracellular signal-regulated kinase (ERK) cascade, which preferentially signals mitogenesis, and the stress-activated protein kinase (SAPK) cascade, which is linked to growth arrest and/or cellular inflammation. In concurrent experiments utilizing rat glomerular mesangial cells (MCs), we dem-

onstrate that growth factors or sphingosine activate ERK but not SAPK. In contrast, inflammatory cytokines or cell-permeable ceramide analogues activate SAPK but not ERK. Ceramide, but not sphingosine, induces interleukin-6 secretion, a marker of an inflamed phenotype. Moreover, ceramide can suppress growth factor- or sphingosine-induced ERK activation as well as proliferation. These studies implicate sphingolipid metabolites as opposing regulators of cell proliferation and inflammation through activation of separate kinase cascades.

INTRODUCTION

Activation of extracellular signal-regulated kinase (ERK) and stress-activated protein kinase (SAPK) cascades culminate in opposing cellular phenotypes. ERKs function predominantly as mediators of growth factor- or phorbol ester-induced cellular proliferation [1,2], whereas SAPKs (also known as Jun kinase or JNK) are involved in regulation of cytokine-induced cellular differentiation, apoptosis or growth arrest [3]. Although small-molecular-mass GTP-binding proteins have been shown to be a common element upstream from both ERK (Ras) and SAPK (Rac) [4–7], the understanding of the physiologically relevant second messengers that control or provide specificity for these discrete signalling pathways is not clear. Sphingolipid metabolites have been shown to influence kinases and phosphatases both in intact cell and cell-free systems [8–11]. Sphingomyelin, the major membrane sphingolipid, can be hydrolysed to form ceramide and then de-acylated to generate sphingosine [12]. Cytokine-induced differentiation or apoptosis has been linked to ceramide generation [12,13], whereas, growth-factor-mediated cell proliferation has been associated with sphingosine formation [14–16]. We have previously demonstrated that growth-factor receptors, but not inflammatory cytokine receptors, can activate ceramidase to form sphingosine at the expense of ceramide [15]. We have used an inhibitor of ceramidase (*N*-oleoyl-ethanolamine) to block platelet-derived growth factor (PDGF)-stimulated DNA synthesis, implicating sphingosine as a component of the mitogenic response of PDGF [15]. The role of ceramide and sphingosine to differentially regulate SAPK and ERK cascades and, in part, to determine the cellular phenotype has been therefore hypothesized.

EXPERIMENTAL

Materials

All sphingolipid metabolites were purchased from either Matreya (Pleasant Gap, PA, U.S.A.) or Serdary (London, Ontario, Canada). Sphingolipid metabolites were dissolved in 5% (w/v) DMSO/95% (v/v) PBS containing 1.0% (w/v) BSA before a 1/100 dilution into Dulbecco's modified Eagle's cell culture medium. All required cell culture media were obtained from Life Technologies (Bethesda, MD, U.S.A.). Primary rat mesangial cell (MC) strains were cultured as previously described [17]. The anti-ERK and -SAPK antibodies were obtained from Santa Cruz Pharmaceuticals (Santa Cruz, CA, U.S.A.). Anti-SAPK1 antibody, also known as anti-JNK1, recognizes a p46 protein and shows no cross-reactivity with a p54 SAPK protein. Anti-ERK2 antibody recognizes the p42, and not the p44, form of ERK. Growth factors and inflammatory cytokines were purchased from either Upstate Biotechnology (Lake Placid, NY, U.S.A.) or CalBiochem (Los Angeles, CA, U.S.A.). Endothelin-1 (ET-1) was purchased from Peptide Institute (Tokyo, Japan). [³H]Thymidine was obtained from DuPont-NEN (Boston, MA, U.S.A.).

Phosphorylation of ERK2 and SAPK1 on tyrosine

Cultured MCs, after 3–10 passages, at 80% confluence were rendered quiescent in supplemented serum- and insulin-free Dulbecco's modified Eagle's medium [17]. Treated MCs were lysed on ice with 500 µl of a buffer containing 25 mM Hepes, pH 7.55, 100 mM NaCl, 20 mM β-glycerophosphate, 1.5 mM MgCl₂, 1 mM vanadate, 0.5 mM EGTA, 0.25 mM EDTA, 0.1% (w/v) Nonidet P-40, 1 mM PMSF and 10 µg/ml each leupeptin/

Abbreviations used: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; SAPK, stress-activated protein kinase; MC, mesangial cell; IL, interleukin; GST, glutathione S-transferase; TNF, tumour necrosis factor α; PDGF, platelet-derived growth factor-ββ; ET, endothelin-1; C₆-ceramide, *N*-hexanoyl-D-erythro-sphingosine.

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pepstatin/aprotinin. From the same pre-cleared cell lysates (150 μ g of protein), either ERK2 or SAPK1 was immunoprecipitated with 0.5 μ g of specific polyclonal anti-ERK2 or anti-SAPK1 antibodies. The formed immunocomplexes were subsequently collected with goat anti-rabbit IgG-agarose. Immunoprecipitated proteins were resolved by SDS/PAGE (12% gels) and transferred to nitrocellulose. Membranes were then immunoblotted with monoclonal anti-phosphotyrosine antibodies conjugated to horseradish peroxidase (ICN, Costa Mesa, CA, U.S.A.) at 1:10000 dilution, and bands were detected with enhanced chemiluminescence.

In Vitro immunoprecipitation kinase assay for ERK2 and SAPK1

Phosphorylation assays were performed on immunoprecipitated proteins in 50 μ l of a kinase buffer (50 mM Hepes, pH 7.55, 100 mM KCl, 25 mM β -glycerophosphate, 10 mM MgCl₂, 1 mM orthovanadate and 0.5 mM EGTA) in the presence of 1 μ Ci of [γ -³²P]ATP (10 mCi/nmol) and 20 μ M unlabelled ATP per reaction for 20 min at 30 °C. The reactions were terminated by the addition of 25 μ l of 3 $\times$ Laemmli buffer, and then the samples were heated at 95 °C for 5 min. Phosphorylated proteins were resolved by SDS/PAGE (12% gels), revealed by autoradiography and quantified by scintillation spectroscopy.

Interleukin (IL)-6 immunofluorescence

IL-6 expression by human tracheal epithelial cells (9HTE) was assessed by specific immunofluorescent staining [18]. Briefly, 9HTE monolayers grown on 8-well LabTech[®] slides were fixed with paraformaldehyde (4% w/v) for 60 min, permeabilized with Triton X-100 (0.1% w/v), then washed extensively with PBS. Monolayers were reacted with a monoclonal antibody to IL-6 at 4 °C for 16 h and then counterstained with an F(ab')₂ FITC conjugated anti-mouse secondary antibody. Specificity of staining was confirmed by blocking IL-6 staining with recombinant IL-6 at 1 μ g/ml.

MC proliferation

Proliferation of quiescent MCs was evaluated by [³H]thymidine uptake into acid-insoluble DNA as previously reported [15]. MCs were first pretreated with *N*-hexanoyl-D-erythro-sphingosine (C₆-ceramide) or vehicle for 16 h before stimulation with sphingosine or PDGF for an additional 24 h. During the last 6 h of this stimulation, the cells were pulsed with 1 μ Ci/ml [³H]thymidine.

RESULTS AND DISCUSSION

Sequential sphingomyelin catabolic metabolites induce opposing biological effects; ceramides suppress mitogenesis and sphingosines enhance cell growth. We hypothesize that this functional dichotomy reflects differential regulation of ERK and SAPK activities by sphingosine and ceramide respectively. Initial experiments were designed to evaluate the effects of sphingosine and cell-permeable ceramide analogues upon ERK2 and SAPK1 activation in intact MCs, smooth-muscle-like pericytes. We have evaluated tyrosine phosphorylation of immunoprecipitated 42 kDa ERK2 and 46 kDa SAPK1 (Figure 1) as well as reconstituted kinase activity as assessed by myelin basic protein [19] and recombinant glutathione S-transferase (GST)-Jun [1] substrate phosphorylation (Figure 2). Similar results were obtained with either procedure. Stimulation of MCs with sphingosine induced ERK2 tyrosine phosphorylation as well as a 7.5-fold increase in ERK2 bioactivity. Synthetic D-(+)-erythro-2-amino-4-*trans*-octadecene-1,3-diol, a stereoisomer of sphingosine

Figure 1 Growth factors (10 ng/ml) and sphingosine (10 μ M) stimulate ERK2 tyrosine phosphorylation (top), whereas inflammatory cytokines (10 ng/ml) and ceramide analogues (10 μ M) enhance SAPK1 tyrosine phosphorylation (bottom)

MCs were stimulated with different agonists at 37 °C for 15 min before lysis and subsequent immunoprecipitation/immunoblotting [1,17,19]. The first and last lanes (Con) correspond to quiescent cells treated with 1 mg/ml BSA dissolved in PBS in the presence or absence of 0.05% (w/v) DMSO respectively. The positions of molecular-mass markers are shown on the left (kDa). This immunoblot is representative of three separate experiments. Sph, sphingosine; Ery-sph, synthetic D-(+)-erythro-2-amino-4-*trans*-octadecene-1,3-diol; Thr-sph, L-threodihydro-sphingosine; C₆-cer, C₆-ceramide; Dih-cer, dihydro-C₆-ceramide; IGF, insulin-like growth factor.

that mimics the effect of natural sphingosine upon cell proliferation, also was effective in inducing ERK2 activation. However, L-threo-dihydrosphingosine, an enantiomer of sphingosine that lacks the double bond between positions 4 and 5 of the sphingoid backbone, was ineffective in activating ERK2 bioactivity. In the same experiments, SAPK1 tyrosine phosphorylation and bioactivity were also evaluated. Neither sphingosine nor its stereoisomers were able to activate SAPK1. In contrast to sphingosine-stimulated ERK2, C₆-ceramide was unable to activate ERK2 bioactivity above control levels. This cell-permeable ceramide analogue did, however, induce tyrosine phosphorylation on SAPK1 and stimulated a 12-fold increase in SAPK1 bioactivity. Dihydro-C₆-ceramide was ineffective in activating SAPK1. The fact that enantiomers, and dihydro-derivatives, of sphingosines and ceramides do not activate ERK2 and SAPK1 excludes the possibility of non-specific, lipophilic, effects of these sphingolipid-derived metabolites. Also, the acute stimulation of kinase activities suggests that these sphingolipid derivatives readily intercalate into the plasma membrane and exert biological activity. In additional experiments (results not shown), sphingosine and C₆-ceramide specifically activated ERK1 and SAPK2 isoforms respectively.

We next investigated activation of ERK2 and SAPK1 with

Figure 2 Growth factors and sphingosine activate ERK2 (left) whereas cytokines and ceramide analogues activate SAPK1 (right)

ERK2 bioactivity was assessed with 10 μ g of myelin basic protein as 32 P-phosphorylation substrate, and SAPK1 bioactivity was determined with 0.5 μ g of GST-Jun (amino acids 5–89) as substrate in this *in vitro* immunoprecipitation kinase assay [1,19]. These experiments were run under identical conditions as those experiments depicted in Figure 1. Molecular-mass markers are shown on the left of each top panel (kDa). All immunoblots are representative of three separate experiments, and the cumulative data are presented as means $\pm$ S.E.M. Con, control; Sph, sphingosine; Ery-sph, synthetic α -(+)-erythro-2-amino-4-*trans*-octadecene-1,3-diol; Thr-sph, L-threodihydrosphingosine; C6-cer, C₆-ceramide; Dih-cer, dihydro-C₆-ceramide; IGF, insulin-like growth factor.

physiological agonists that selectively induce either ceramide or sphingosine formation. We have previously reported that inflammatory cytokines, including IL-1 α and tumour necrosis factor α (TNF), enhance sphingomyelinase activity, but not ceramidase activity, *in vitro*, leading to accumulation of ceramide, exclusively [15]. As depicted in Figures 1 and 2, IL-1 and TNF, known activators of SAPK and inducers of ceramide, stimulated a 12-fold increase in SAPK bioactivity without affecting ERK activation. In contrast, growth factors, including PDGF, preferentially stimulate ceramidase activity to form sphingosine at the expense of ceramide [15]. Thus PDGF and insulin-like growth factor mimicked the effect of sphingosine to stimulate ERK but not SAPK (Figure 1). The observation that PDGF activates ERK, but not SAPK, cascades has also been demonstrated in NIH 3T3 fibroblasts [20]. ET, a G-protein-coupled proliferative peptide for MCs, also activated ERK but not SAPK (Figures 1 and 2). In contrast to PDGF, ET does not elevate endogenous sphingosine nor do inhibitors of ceramidase reduce ET-stimulated proliferation [15]. This suggests that ET-activated ERK and subsequent cell proliferation occurs independent of sphingosine formation. Moreover, these data may suggest that tyrosine kinase receptors (PDGF) and serpentine receptors (ET) utilize separate pathways coupled to ERK activation. It should be noted that the dose of PDGF that stimulated maximal MC proliferation (0.4 nM) is 250-fold less than the maximal proliferative dose for ET (100 nM). Thus tyrosine kinase receptors may utilize this sphingosine-activated ERK cascade as a mechanism to further augment the proliferative response.

We evaluated time- and dose-response curves for sphingosine-induced activation of ERK2 and C₆-ceramide-induced stimulation of SAPK1 in MCs (Figure 3). We assessed both tyrosine phosphorylation (results not shown) and *in vitro* kinase activity. The time and dose responses were very similar for sphingosine-stimulated ERK and ceramide-activated SAPK and concur with studies that utilize exogenous sphingolipid metabolites to regulate

nuclear transcriptional events [21–23]. Exogenous sphingosine (10 μ M) incubated with intact MCs stimulated immunoprecipitated ERK2 bioactivity as early as 5 min, peaking at 15 min and returning to basal level after 60 min. PDGF and EGF induce ERK bioactivity with a similar time course as sphingosine in MCs ([19]; Y. Wang, unpublished work), and transient ERK activity correlates with mitogenesis, but not differentiation, in PC12 cells [24]. Enhanced immunoprecipitated SAPK bioactivity was observed after a 2 min incubation of intact MCs with 10 μ M C₆-ceramide. Peak SAPK activity was observed after a 10 min incubation. Compared with sphingosine-stimulated ERK bioactivity, it appears that peak ceramide-enhanced SAPK bioactivity can be maintained for a longer period of time. In terms of dose, sphingosine initiates ERK2 activation and C₆-ceramide induces SAPK activation at a threshold concentration of 0.1 μ M, with peak responses observed at 10 μ M (sphingosine) and 100 μ M (C₆-ceramide).

We next asked if sphingolipid-derived metabolites can directly serve as cofactors for ERK in a cell type where acute stimulation of ERK with growth factors leads to a proliferative response [24]. Immunoprecipitated p42 ERK2 bioactivity from either EGF-treated or control PC12 cells was assessed *in vitro* in the presence or absence of sphingolipid (Table 1). Ceramide or sphingosine had no significant effect upon either agonist-stimulated or basal ERK activity. These results suggest that the action of sphingosine to regulate the ERK cascade resides upstream of ERK itself.

As sphingosine and ceramide differentially activate ERK and SAPK cascades, we next asked if these sphingolipid metabolites selectively alter the cellular phenotype. Thus we investigated IL-6 secretion, a hallmark of an inflamed, stress-activated, cellular phenotype to dissociate ceramide-activated SAPK from sphingosine-activated ERK pathways (Table 2). Cultured MCs and human tracheal epithelial (9HTE) cells secrete IL-6 in response to stresses that activate SAPK [25,26]. C₆-ceramide mimicked the effect of IL-1 to induce IL-6 production. Blocking experiments with recombinant IL-6 reduced both IL-1- and

Figure 3 Sphingosine induces ERK2 bioactivity and C_6 -ceramide stimulates SAPK1 bioactivity in a time- (top) and dose- (bottom) dependent manner

Sphingosine-induced ERK2 activity was assessed by [32 P]phosphate incorporation into myelin basic protein, whereas SAPK1 activity was determined by [32 P]phosphate incorporation into recombinant GST-Jun, in an *in vitro* kinase reaction utilizing immunoprecipitated ERK or SAPK. All immunoblots are representative of three separate experiments, and the cumulative data are presented as means $\pm$ S.E.M.

Table 1 Neither sphingosine nor C_6 -ceramide alters ERK2 activity in cell lysates derived from either EGF-treated (50 ng/ml) or control PC12 cells

Immunoprecipitated ERK2 activity was assessed as described in Figure 1 except that either ceramide, sphingosine or vehicle was added to the *in vitro* kinase reaction. $n = 3$ experiments in duplicate; data are means $\pm$ S.E.M.

	ERK2 activity (d.p.m./well)		
	Vehicle	Sphingosine	C_6 -Ceramide
Control	2569 $\pm$ 238	2613 $\pm$ 196	2382 $\pm$ 216
EGF	7124 $\pm$ 565	7810 $\pm$ 427	6843 $\pm$ 386

ceramide-induced IL-6 immunofluorescence to the control value. Even though lung epithelial cells express receptors for PDGF [27], both sphingosine and PDGF failed to induce IL-6 production.

We, as well as others, have demonstrated that sphingosine is a mitogenic signal used by growth-factor-receptor tyrosine kinases [15,16]. As ceramide arrests the cell cycle at G_0/G_1 [12,13], we investigated if C_6 -ceramide and subsequent SAPK activity reduces proliferation by affecting sphingosine- or PDGF-stimulated ERK bioactivity. We assessed the chronic effects of

Table 2 C_6 -ceramide, but not sphingosine, stimulates IL-6 secretion in 9HTE cells

Data from three experiments are presented as a percentage of immunofluorescent cells (epi-fluorescent microscopy) per total cells (bright field microscopy) within each microscopic field. Sphingolipids (1 μ M) or agonists (10 ng/ml) were added for 16 h before cells were prepared for immunofluorescence. Data are means $\pm$ S.E.M. N.D., not determined. r, recombinant.

	IL-6 production (% cells immunofluorescent)				
	Vehicle	C_6 -Ceramide	IL-1	Sphingosine	PDGF
Without rIL-6	2.2 $\pm$ 0.5	32.2 $\pm$ 4.0	31 $\pm$ 1.9	0.1 $\pm$ 0.1	0.3 $\pm$ 0.2
With rIL-6	N.D.	13 $\pm$ 8.0	3 $\pm$ 0.3	N.D.	N.D.

C_6 -ceramide pretreatment (16 h) upon sphingosine- and PDGF-mediated cellular proliferation (Figure 4, upper panel) and ERK activity (Figure 4, lower panel). Sphingosine and PDGF induced a 7-fold and a 12.5-fold increase, respectively, of [3 H]thymidine uptake into MC DNA. Sphingosine and PDGF had an additive effect upon DNA synthesis. C_6 -ceramide inhibited both sphingosine- and PDGF-stimulated DNA synthesis by 100% and 75% respectively. The additive effects of PDGF and sphingosine on cell proliferation were suppressed 60% by ceramide pretreatment. These data suggest that both sphingosine and PDGF mediate proliferation via a similar ERK-dependent signalling pathway. Confirming these thymidine uptake experiments, continuous C_6 -ceramide pretreatment for 16 h abolished sphingosine-induced ERK bioactivity. Also, PDGF-stimulated ERK activity, in the presence or absence of exogenous sphingosine, was partially inhibited by chronic C_6 -ceramide preincubation. In results not shown, MCs acutely pre-exposed to C_6 -ceramide for 1 h still responded to sphingosine or PDGF with an ERK-dependent proliferative response. These findings suggest that chronic, but not acute, SAPK activation may be sufficient to attenuate subsequent agonist-stimulated ERK activation and mitogenesis. These cross-inhibition studies suggest that activated SAPK initiates downstream events that are subsequently able to inhibit ERK-mediated cell proliferation. Thus for both cellular proliferation and cellular inflammation we have dissociated ERK and SAPK signalling cascades as consequences of growth-factor-receptor-induced sphingosine formation and cytokine-receptor-induced ceramide generation.

The above studies suggest a scenario by which SAPK and ERK cascades are parallel pathways resulting in induction of cell inflammation and cell proliferation respectively. In addition, activation of SAPK and inactivation of ERK cascades are critical events for induction of apoptosis [28]. Recent studies confirm the actions of sphingolipid metabolites on selective MAPK cascades. C_2 -ceramide mimics the effects of TNF to preferentially activate SAPK in HL-60 cells [24]. Ceramide pretreatment also diminishes basal ERK activity [24]. Sphingosine or sphingosine-1-phosphate stimulates the ERK cascade and enhances activator protein-1/DNA binding activity [23,29]. We now demonstrate functional implications for differential activation of MAPK cascades by sphingolipid derivatives.

We have demonstrated the opposing actions of ceramide and sphingosine upon cellular function in mesenchymal and epithelial cell types. However, significant cross-talk between sphingosine- and ceramide-stimulated kinase pathways can still occur. In HL-60 and Swiss 3T3 cells, ceramide analogues [30] as well as TNF [30,31] phosphorylate 42 and 44 kDa proteins that may be ERK. However, other tyrosine-phosphorylated proteins at 46 kDa and 55 kDa that may reflect SAPK1 or SAPK2 were also phosphorylated by ceramide [30]. This cross-talk mechanism

Figure 4 C₆-Ceramide pre-exposure reduces PDGF- and sphingosine-stimulated proliferation (top) and ERK2 bioactivity (middle and bottom) in MCs

MCs pretreated with 10 μ M C₆-ceramide or vehicle for 16 h were subsequently stimulated with 10 μ M sphingosine or 10 ng/ml PDGF for 24 h (top) or 15 min (middle and bottom). Proliferation was assessed by [³H]thymidine uptake into acid-insoluble DNA. ERK2 bioactivity was evaluated by assessing [³²P]phosphate incorporation into myelin basic protein in an *in vitro* kinase reaction as described above. Molecular-mass markers are shown on the left of the middle panel (kDa). *n* = 3 or 4; data are means $\pm$ S.E.M. Con, control; Sph, sphingosine; C6-cer, C₆-ceramide.

may involve a partially purified protein kinase that, upon TNF activation, phosphorylates Raf and subsequently the ERK cascade [32]. Also, culture conditions may determine the actions of sphingolipid metabolites. In quiescent cells, sphingosine is a mitogen whereas in phorbol 12-myristate 13-acetate-differentiated HL-60 cells sphingosine or sphinganine blocks cell adherence and growth arrest [33] or induces apoptosis [34].

In conclusion, growth-factor-receptor-induced proliferation

may, in part, be mediated by sphingosine-regulated ERK bioactivity. In contrast, inflammatory cytokine receptors may signal growth arrest and induce an inflamed phenotype through a ceramide-regulated SAPK cascade. This study establishes sphingomyelin-derived metabolites as key mediators of cellular proliferation and inflammation via activation of discrete protein kinase cascades.

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REFERENCES

- Davis, R. J. (1994) Trends Biochem. Sci. **19**, 470–474
- Cobb, M. H., Robbins, D. J. and Boulton, T. G. (1991) Curr. Opin. Cell. Biol. **3**, 1025–1032
- Sluss, H. K., Barrett, T., Derijard B. and Davis R. J. (1994) Mol. Cell. Biol. **14**, 8376–8384
- Yan, M., Dai, T., Deak, J. C., Kyriakis, J. M., Zon, L. I., Woodgett, J. R. and Templeton D. J. (1994) Nature (London) **372**, 798–800
- Coso, O. A., Chiarello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T. and Gutkind J. S. (1995) Cell **81**, 1137–1146
- Minden, A., Lin, A., Claret, F. X., Abo, A. and Karin, M. (1995) Cell **81**, 1147–1157
- Olson, M. F., Ashworth, A. and Hall, A. (1995) Science **269**, 1270–1272
- Joseph, C. K., Byun H, Bittman, R. and Kolesnick, R. N. (1993) J. Biol. Chem. **268**, 20002–20006
- Dobrowsky, R. T., Kamibayashi, C., Mumby, M. C. and Hannun, Y. A. (1993) J. Biol. Chem. **268**, 15523–15530
- Snufferlein, T. and Rozengurt, E. (1994) J. Biol. Chem. **269**, 27610–27617
- Wu, J., Spiegel, S. and Sturgill, T. W. (1995) J. Biol. Chem. **270**, 11484–11488
- Hannun, Y. A. (1994) J. Biol. Chem. **269**, 3125–3128
- Jayadev, S., Liu, B., Bielawaska, A. E., Lee, J. Y., Nazaire, F., Pushkareva, M. Y., Obeid, L. M. and Hannun, Y. A. (1995) J. Biol. Chem. **270**, 2047–2052
- Jacobs, L. and Kester, M. (1993) Am. J. Physiol. **265**, C740–C747
- Coroneos, E., Martinez, M., McKenna S. and Kester, M. (1995) J. Biol. Chem. **270**, 23305–23309
- Olivera, A. and Spiegel, S. (1993) Nature (London) **365**, 557–559
- Kester, M., Coroneos, E., Thomas, P. J. and Dunn, M. J. (1994) J. Biol. Chem. **269**, 22574–22580
- Bonfield, L., Konstan, M. W., Burfeind, P., Panuska, J. R., Hilliard, J. B. and Berger, M. (1995) Am. J. Resp. Cell Mol. Biol. **13**, 257–261
- Wang, Y., Simonson, M. S., Pouyssegur, J. and Dunn, M. J. (1992) Biochem. J. **287**, 589–594
- Coso O. A., Chiariello, M., Kalinec, G., Kyriakis, J. M., Woodgett, J. and Gutkind, S. (1995) J. Biol. Chem. **270**, 5620–5624
- Marshall, C. J. (1995) Cell **80**, 179–185
- Wolff, R. A., Dobrowsky, R. T., Bielawaska, A., Obeid, L. M. and Hannun, Y. A. (1994) J. Biol. Chem. **269**, 19605–19609
- Su, Y., Rosenthal, D., Smulson, M. and Spiegel, S. (1994) J. Biol. Chem. **269**, 16512–16517
- Westwick, J. A., Bielawaska, A. E., Dbaibo, G., Hannun, Y. A. and Brenner, D. A. (1995) J. Biol. Chem. **270**, 22689–22692
- Zoya, C., Wang, J. M., Bettoni, S., Sironi, M., Renzi, D., Chiaffarino, F., Abboud, H. E., VanDamme, J., Mantovani, A. and Remuzzi, G. (1991) Am. J. Physiol. **138**, 991–1003
- Noah, T. L. and Becker, S. (1993) Am. J. Physiol. **265**, L472–L478
- Caniggia, I., Liu, J. Han, R., Buch, S., Funa, K., Tanswell, K. and Post, M. (1993) Am. J. Resp. Cell Mol. Biol. **9**, 54–63
- Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J. and Greenberg, M. E. (1995) Science **269**, 1270–1272
- Wu J., Spiegel, S. and Sturgill, T. W. (1995) J. Biol. Chem. **270**, 11484–11488
- Raines, M. A., Kolesnick, R. and Golde, D. W. (1993) J. Biol. Chem. **268**, 14572–14575
- Van Lint, J., Agostinis, P., Vandevoorde, V., Haegeman, G., Fiers, W., Merlevede, W. and Vandenheede, J. R. (1992) J. Biol. Chem. **267**, 25916–25921
- Yao, B., Zhang, Y., Delikat, S., Mathias, S., Basu, S. and Kolesnick, R. (1995) Nature (London) **378**, 307–310
- Merrill, A. H., Sereni, A. M., Stevens, V. L., Hannun, Y. A., Bell, R. M. and Kinkaade J. M. (1986) J. Biol. Chem. **261**, 12610–12615
- Ohta, H., Sweeney, E. A., Masamune, A., Yatomi, Y., Hakamori, S. and Igarashi, Y. (1995) Cancer Res. **55**, 691–697