Sphingosine 1-Phosphate (S1P) Receptors 1 and 2 Coordinately Induce Mesenchymal Cell Migration through S1P Activation of Complementary Kinase Pathways*

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Background: Coupling of bone degradation to subsequent bone formation requires recruitment of osteoblast precursors.

Results: Osteoclasts produce sphingosine 1-phosphate (S1P), which stimulates mesenchymal (skeletal) stem cell migration by activating kinase signaling pathways.

Conclusion: Coupling of bone resorption to bone formation involves S1P-mediated recruitment of osteoblastic cells.

Significance: Enhancing kinase signaling in osteoblastic cells may be a novel approach to enhance bone formation.

Normal bone turnover requires tight coupling of bone resorption and bone formation to preserve bone quantity and structure. With aging and during several pathological conditions, this coupling breaks down, leading to either net bone loss or excess bone formation. To preserve or restore normal bone metabolism, it is crucial to determine the mechanisms by which osteoclasts and osteoblast precursors interact and contribute to coupling. We showed that osteoclasts produce the chemokine sphingosine 1-phosphate (S1P), which stimulates osteoblast migration. Thus, osteoclast-derived S1P may recruit osteoblasts to sites of bone resorption as an initial step in replacing lost bone. In this study we investigated the mechanisms by which S1P stimulates mesenchymal (skeletal) stem cells to respond to S1P with a chemotactic response (9–14). Our results demonstrate that the chemokine S1P couples bone formation to bone resorption through activation of kinase signaling pathways.

During longitudinal growth, osteoclast-mediated bone resorption is required to expand the marrow cavity whereas one formation by osteoblasts creates larger bones. In adults, the cycle of resorption and formation repairs damaged bones and modulates systemic and local calcium needs. In young adults, the rate of bone resorption and subsequent bone formation is tightly controlled in that resorbed bone is precisely replaced in both location and amount. This concept has been termed coupling (1, 2). With aging, coupling becomes unbalanced such that the increase in osteoclast numbers and activity cannot be met with equal bone formation, resulting in a net loss of bone. Because age-related bone loss is such a prevalent occurrence that encompasses nearly half of the human population worldwide, it is crucial to determine the mechanisms by which bone formation is linked to bone resorption to design the most effective therapies to prevent uncoupling.

A necessary early step in coupling is the recruitment of osteoblast progenitors to the bone surface through stimulating their migration to bone. We documented that osteoclasts secrete sphingosine 1-phosphate (S1P)2 (3). S1P present in conditioned medium from cultured osteoclasts stimulates random cell movement (chemokinesis) in mesenchymal cells (3). S1P was also found to stimulate directed migration of cancer cells, including prostate, myeloma, thyroid, and breast (4–8). Many nontransformed cells in the body such as endothelial cells, stem cells, B cells, T cells, muscle cells, and dendritic cells, and osteoclast precursors respond to S1P with a chemotactic response (9–14). We therefore investigated whether osteoclast S1P is chemotactic for mesenchymal (skeletal) stem cells (MSCs) and the mechanisms by which S1P stimulates MSC migration.

EXPERIMENTAL PROCEDURES

Unless otherwise indicated, all chemicals were from Sigma. Osteoclast Culture and Conditioned Media Preparation—Six- to 8-week-old C57BL/6 mice (Jackson Laboratories) were killed, and bone marrow was harvested as we have reported previously (3). Red blood cells were lysed, and the remaining bone marrow cells were cultured in α-Minimal Essential Medium (αMEM) supplemented with 10% (v/v) fetal bovine serum (FBS).

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1 The abbreviations used are: S1P, sphingosine 1-phosphate; DMSO, dimethyl sulfoxide; FAK, focal adhesion kinase; GTPγS, guanosine 5′-3-O-(thio)triphosphate; hMSC, human MSC; hTERT, human telomerase reverse transcriptase; MSC, mesenchymal (skeletal) stem cell; S1PR, S1P receptor; SPHK, sphingosine kinase.

2 The abbreviations used are: S1P, sphingosine 1-phosphate; DMSO, dimethyl sulfoxide; FAK, focal adhesion kinase; GTPγS, guanosine 5′-3-O-(thio)triphosphate; hMSC, human MSC; hTERT, human telomerase reverse transcriptase; MSC, mesenchymal (skeletal) stem cell; S1PR, S1P receptor; SPHK, sphingosine kinase.
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...serum (FBS) and 25 ng/ml macrophage colony-stimulating factor (M-CSF) and incubated at 37 °C, in 5% CO₂. One day after isolation, adherent mesenchymal cells were discarded, and the nonadherent cells were cultured in 100 ng/ml receptor activator of NF-κB (RANKL) and 25 ng/ml M-CSF for 4 days with a refeeding on day 3. For migration assays, mature osteoclast-conditioned media were harvested and centrifuged to remove cell debris. The conditioned media were stored at −80 °C until assayed. All protocols were approved by the Mayo Clinic IACUC prior to the start of the studies.

MSC Culture and Migration Assay—Human bone marrow-derived MSCs overexpressing human telomerase reverse transcriptase (hMSC-TERT) were employed as a model for hMSC. Development and characterization of this cell line have been described previously (15, 16).

 Cultures were maintained in αMEM supplemented with 10% FBS (base medium). For migration experiments, 95% confluent cells were harvested and assayed using the QCM 24-well Colorimetric Cell Migration Assay (Millipore). hMSC-TERT cells were plated in 100-mm tissue culture dishes. When 95% confluent, cells were rinsed with PBS and RNA harvested at times indicated in the figure legends using the RNeasy micropurification kit (Qiagen). cDNA was synthesized, and real time PCR analysis was performed.

Quantitative Real Time Polymerase Chain Reaction—Cells were rinsed with PBS and RNA harvested at times indicated in the figure legends using the Qiagen micropurification kit (Qiagen) according to the product literature. Following quantification, cDNA was synthesized, and real time PCR analysis was carried out as we have reported (3). Primers were: S1PR1 forward, 5’-GGAGCCCCTAGAGGAGAGAGTGT-3’ and reverse, 5’-GGGACTGCGGAGAGGACTGAGTG-3’; tubulin A1A forward, 5’-GAGTGATCTCTCCACTCCAAGTCGTT-3’ and reverse, 5’-TAGAGCTCCAGCGAGGCTT-3’.

 Messenger RNA levels were calculated using the ΔΔ-Ct method (3).

Rho GTPase Family Activation Assay—hTERT cells were plated in 6-well plates. When 95% confluent, wells containing base medium were treated with vehicle (1 μl/ml 5% acidified DMSO in H₂O) or the S1P agonist. Other wells were switched to osteoclast-conditioned medium and treated with vehicle or combined S1PR1 and S1PR2 receptor antagonist for 10 min at 37 °C. Cells were processed and assayed for RhoA, Rac1, and Cdc42 activity using a kit from Cell Biolabs according to the instructions. Briefly, cell lysates were incubated with agaroose beads coupled to either Rhotekin Rho binding domain peptide (RhoA assay) or p21 binding domain of p21-activated protein kinase (Rac1 and Cdc42 assays). Separate aliquots of lysate were incubated for 30 min at 30 °C with nonhydrolyzable GTPγS to activate all GTPases prior to mixing with the agaroose beads. After incubation on ice for 60 min, the beads were spun down and repeatedly washed. The agaroose beads were suspended in Western blot sample buffer, and the presence of bound activated GTPases was evaluated by Western blotting with specific antibodies to the GTPases.

Western Blot Analysis—hMSC-TERT cells were plated in 100-mm tissue culture dishes. When 95% confluent, cells were rinsed three times with phosphate-buffered saline and serum-starved for 6 h in αMEM with 1% bovine serum albumin. Cells were treated with the indicated substances for the indicated time as detailed in the figure legends using reagents detailed above for migration assays. Cell extracts were harvested and protein concentrations determined with the Bio-Rad DC Protein Assay kit as instructed. Proteins (40 μg) were separated using 10% SDS-PAGE followed by electroblotting to Immobilon-P membranes (Millipore). Membranes were probed as described with antibodies to mouse phosphorylated signaling antibodies (3). All antibodies were from Cell Signaling. Lane loading was monitored by reprobing blots for tubulin (University of Iowa Hybridoma Bank). Signals were visualized using the ECL Plus detection system (Amersham Biosciences) according to the manufacturer’s instructions.

Statistics—Each experiment had at least three replicates and was repeated at least three times. These results are representative of the repeats. Data were analyzed using a one-way analysis of variance compared with controls as indicated in each figure legend and are presented as mean ± S.E. Significance was determined at p < 0.05 using KaleidaGraph software (Synergy Software, Reading PA).

RESULTS

Osteoclasts Secrete S1P to Promote Chemotaxis of Mesenchymal Cells—Coupling requires recruitment of osteoprogenitors to the location of bone resorption through chemotaxis, or directed migration. Previously, we showed that osteoclasts promote MSC chemokinesis and that movement was reduced with an antagonist the blocks S1P-receptor interactions (3). Here we investigated whether secreted S1P induces MSC chemotaxis.
Osteoclast-conditioned medium induced MSC chemotaxis and S1P-receptor antagonists blocked this response (Fig. 1A). MSC responded to a S1P agonist added to the base medium with increased migration, confirming that S1P is a chemotactic agent for mesenchymal cells (Fig. 1B). We examined expression of the three S1P receptors S1PR1, S1PR2, and S1PR3 in MSC cultures (Fig. 2). As cultures reach confluence, S1PR1 and S1PR2 expression increased significantly (Fig. 2A). S1PR3 expression was highly variable. We employed receptor-selective antagonists to resolve which of these was involved in the mesenchymal response (Fig. 2B). Blocking either S1PR1 or S1PR2 reduced S1P migration stimulation, and combining S1PR1 and S1PR2 antagonists further reduced migration to base media levels. We did not detect any role for S1P3 in mediating S1P influences on migration (data not shown).

Rho GTPase and Kinase Signaling Involvement in S1P-induced Migration of Mesenchymal Cells—S1P receptors are G protein-coupled receptors that activate several GTPases (for review, see Ref. 17). To determine how S1P promoted MSC chemotaxis, the Rho GTPase family was evaluated (Fig. 3). RhoA was rapidly activated in MSC cultured in base medium containing the S1P agonist or cultured with osteoclast-conditioned media (Fig. 3A, second and third lanes). Antagonists of S1PR1 and S1PR2 combined reduced conditioned media mediated RhoA activation (Fig. 3A, fourth lane). We were unable to detect any S1P activation of Rac1 or Cdc42 (data not shown).
To determine the role of RhoA GTPase in S1P stimulation of migration, mesenchymal cells were treated with a selective RhoA inhibitor prior to assessing mesenchymal cell migration in response to S1P agonist (Fig. 3B). Rho inhibition did not alter mesenchymal cell migration in response to S1P.

Another key mediator of migration that is activated by S1P is FAK (for review, see Ref. 18), which is an upstream activator of the PI3K/AKT signaling pathway (for review, see Ref. 19). We therefore examined S1P influences on FAK/AKT activation and observed rapid activation of both FAK and AKT (Fig. 4A).

Because the JAK/STAT pathway has been implicated in migration, we also examined mesenchymal S1P responses for evidence of activation of this pathway (20). We observed rapid phosphorylation of JAK1 and STAT3 in response to S1P treatment (Fig. 4A). We did not detect phosphorylation of JAK2, JAK3, or STAT5 (data not shown). Inhibition of AKT, FAK, PI3K, JAK, or STAT3 blocked S1P-induced migration of mesenchymal cells (Fig. 4B).

S1PR1 and S1PR2 Coordinate Activation Kinase Signaling Pathways (Summarized in Fig. 8)—To investigate the mechanisms of pathway activation, we co-treated mesenchymal cells with the S1P agonist and receptor-selective antagonists (Fig. 5).

Based on our results documenting that S1P activated S1PR1 and S1PR2, but not S1PR3, we surmised that co-treatment with S1P and blocking S1PR2 would allow activation of only S1PR1 whereas blocking S1PR1 would allow activation of only S1PR2. S1PR2 antagonists blocked phosphorylation of FAK and AKT, indicating that S1PR1 activated JAK/STAT signaling (Fig. 5A).

In contrast, S1PR1 antagonists blocked phosphorylation of JAK1 and STAT3, supporting S1PR2 activation of FAK/PI3K/AKT signaling (Fig. 5B). Inhibiting FAK, PI3K, or AKT had no impact on migration when S1PR1 was activated by S1P, but blocking JAK or STAT3 inhibited the migratory response (Fig. 6A). Blocking AKT, FAK, or PI3K inhibited migration stimulated by S1PR2 whereas blocking either JAK or STAT3 had no impact on the migratory response to S1P (Fig. 6B). JAK has been reported to activate the PI3K/AKT pathway in cancer cells (21).

To determine whether FAK was upstream or downstream of PI3K and AKT signaling, the ability of constitutively active PI3K to overcome targeted pathway deletion was analyzed (Fig. 7A). Activated PI3K overcame FAK inhibition but was unable to overcome inhibition of AKT, JAK, or STAT3. To evaluate whether there was cross-talk between signaling pathways in mesenchymal cells in response to S1P, the ability of constitutively active AKT to overcome targeted pathway activation was evaluated (Fig. 7B). Constitutively active AKT overcame FAK or PI3K inhibition, but was unable to overcome inhibition of
either JAK or STAT3. These data demonstrate that JAK/STAT and FAK/PI3K/AKT signaling independently coordinate to promote mesenchymal cell migration in response to S1P.

**DISCUSSION**

Sphingosine kinases (SPHKs) are lipid kinases related to diacylglycerol kinases or ceramide kinases and are evolutionarily conserved from yeast to mammals (22). SPHK1 and SPHK2 generate S1P in cells by the transfer of a phosphate group from ATP to sphingosine. Functionally, these enzymes seemed to be interchangeable in S1P production because mice lacking either of them appear normal and breed normally whereas double knock-out mice die embryonically (23). The enzymes do have unique tissue-specific functions, however, as mice lacking SPHK1, but not mice lacking SPHK2, are more resistant to LPS-induced inflammation and are resistant to the progressive neurodegeneration seen in genetically induced Sandhoff disease (24, 25). At the amino acid level, SPHK1 and SPHK2 are ~50% homologous. Although they both generate S1P from the same substrates, ATP and sphingosine, they exhibit distinct functional differences (26). For example, SPHK1 is more selective in its substrate, and SPHK2 phosphorylates a broader spectrum of sphingolipid-like compounds (27). Our studies demonstrate that osteoclast precursors express higher levels of SPHK1 as they mature, supporting a possible role for SPHK1 in osteoclast-mediated coupling (3).

The SPHKs are G protein-coupled receptors that activate Rho family GTPases, but reports have also documented that they also signaling through other pathways such as the JAK/STAT and PI3K pathways (28, 29). In the studies reported here, mesenchymal cell pathways activated by S1P include RhoA GTPase, FAK/PI3K/AKT, and JAK/STAT. RhoA mediates migration responses downstream of G proteins in many cell types in response to multiple stimuli (30–32). In lymphocytes, S1PR1 activation of both Rac1 and Cdc42 is required for S1P migratory stimulation (33). We examined S1P action of three members of the Rho GTPase family in mesenchymal cells. We found that RhoA but not Rac1 or Cdc42 was rapidly activated by S1P treatment. Given the well documented roles of Rho family members in migration, it was unexpected that RhoA does not mediate S1P migration stimulation. Instead, we uncovered...
distinct roles for S1PR1 and S1PR2 in activating JAK/STAT3 and FAK/PI3K/AKT pathways, respectively. Constitutively active AKT was able to overcome pharmacological inhibition of FAK and PI3K, but not inhibition of JAK or STAT3. These data confirm that these pathways are activated in parallel, and each of these pathways contributes to S1P activation of mesenchymal cell migration (Fig. 8). Kinase-driven signaling regulates migration of many types of cells. The PI3K/AKT pathway controls migration of both normal cells and tumor cells, and deregulation of this pathway has been implicated in driving tumor cell progression (34–38). Components of JAK/STAT signaling including JAK1 and STAT3 are also well documented to modulate cell migration (39–42).

Our data that mesenchymal cells express both S1PR1 and S1PR2 mirror the finding that osteoclast precursors express both receptors (9). Both S1P receptors are essential for the recruitment of osteoclast precursors from circulation, but they function in diametrically opposed ways (9). A subset of circulating osteoclast precursors express high levels of S1PR2. Binding of S1P to S1PR2 causes a chemorepellent response to S1P, unlike the chemotactic response in mesenchymal cells reported here. Because blood has much higher levels of S1P than the bone marrow, high S1P2 expression in circulating osteoclast precursors causes the cells to leave the circulation and enter the bone marrow environment. The interactions between S1P and S1PR2 lead to reduced S1PR2 expression, decreasing the chemorepulsive response. Once S1P binds to S1PR2, S1PR1 expression increases, stimulating a chemotactic response to S1P, leading to osteoclast precursor migration to the bone surface. The chemorepulsion functions of osteoclast precursor S1P/S1PR2 responses is supported by the correlation of high circulating S1P levels with low bone density recently revealed (43). Our studies document that S1PR1 and S1PR2 are also essential for the S1P chemoattractive response in mesenchymal cells. Thus, the chemoattractive influences of local S1P in the bone marrow microenvironment would promote migration of both osteoclast and osteoblast precursors. This may contribute to the increases in both osteoclasts and osteoblasts during high bone turnover.

The mesenchymal cells used in this study are resident bone marrow stromal cells (15). These cells exhibit extensive random movement, or chemokinesis, as documented by the presence of cells that pass through the membrane in the absence of any stimulus. Directed movement stimulation in vivo by S1P would bring the cells close to higher concentrations of differentiation factors such as BMPs and Wnts that we have shown to be secreted by osteoclasts and TGF-β released from the bone matrix by bone resorption (3, 44). Thus, the in vivo significance of stimulating movement toward bone resorbing osteoclasts is likely to be significant. It could be interesting to determine whether S1P influence on circulating osteoprogenitors differs.
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It seems unlikely because our data support that both S1PR1 and S1PR2 are involved in pro-migration responses, unlike osteoclast precursors. S1P stimulates RANKL production by osteoblasts; thus, there is also an indirect influence of S1P to promote osteoclast differentiation, suggesting a positive feedback in which osteoclast S1P production may enhance osteoclast differentiation and survival as well as osteoblast precursor recruitment (25).

Our studies support a role for S1P in recruiting osteoblast precursors and Sato et al. (45) recently documented that S1P enhances osteoblast differentiation responses to BMP2, indicating that S1P promotes anabolic responses through multiple mechanisms. Evaluations of clinical samples, in vitro studies, and in vivo animal models have supported the hypothesis that targeting SPHK/SIP may be beneficial therapeutically. Blocking S1P production is the focus of intense interest due to the link between SPHK1 and cancer, fibrosis, rheumatoid arthritis, and inflammation development (46–58). In contrast to potentiating S1P production is the focus of intense interest due to the insight.

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References

1. Martin, T. J., and Sims, N. A. (2005) Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol. Med. 11, 76–81
2. Karsdal, M. A., Martin, T. J., Bollerslev, J., Christiansen, C., and Henriksen, K. (2007) Are nonresorbing osteoclasts sources of bone anabolic activity? J. Bone Miner. Res. 22, 487–494
3. Pederson, L., Ruan, M., Westendorf, J. J., Khosla, S., and Oursler, M. J. (2008) Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine 1-phosphate. Proc. Natl. Acad. Sci. USA 105, 20764–20769
4. García-Bernal, D., Redondo-Muñoz, J., Dios-Exponera, A., Chèvre, R., Bailón, E., Garaya, M., Arelano-Sánchez, N., Gutierrez, N. C., Hidalgo, A., García-Pardo, A., and Teixido, J. (2013) Sphingosine 1-phosphate activates chemokine-promoted myeloma cell adhesion and migration involving αβ8 integrin function. J. Pathol. 229, 36–48
5. Brocklyn, J. R. (2010) Regulation of cancer cell migration and invasion by sphingosine 1-phosphate. World J. Biol. Chem. 1, 307–312
6. Sekine, Y., Suzuki, K., and Remaley, A. T. (2011) HDL and sphingosine 1-phosphate activate STAT3 in prostate cancer DU145 cells via ERIK1/2 and S1P receptors, and promote cell migration and invasion. Prostate 71, 690–699
7. Bergelín, N., Löf, C., Balthasar, S., Kalhori, V., and Törnquist, K. (2010) S1P1 and VEGFR-2 form a signaling complex with extracellularly regulated kinase 1/2 and protein kinase C-alpha regulating ML-1 thyroid carcinoma cell migration. Endocrinology 151, 2994–3005
8. Sarkar, S., Maceyka, M., Haid, N. C., Paugh, S. W., Sankala, H., Milstien, S., and Spiegel, S. (2005) Sphingosine kinase 1 is required for migration, proliferation and survival of MCF-7 human breast cancer cells. FEBS Lett. 579, 5313–5317
9. Ishii, M., Egen, J. G., Klauschen, F., Meier-Schellersheim, M., Saiki, Y., Vacher, J., Proia, R. L., and Germain, R. N. (2009) Sphingosine 1-phosphate mobilizes osteoblast precursors and regulates bone homeostasis. Nature 458, 524–528
10. Lamana, A., Martin, P., de la Fuente, H., Martínez-Muñoz, L., Cruz-Adalia, A., Ramirez-Huesca, M., Escrivano, C., Gollmer, K., Mellado, M., Stein, J. V., Rodriguez-Fernandez, J. L., Sanchez-Madrid, F., and del Hoyo, G. M. (2011) CD69 modulates sphingosine 1-phosphate-induced migration of skin dendritic cells. J. Invest. Dermatol. 131, 1503–1512
11. Howard, C., Murray, P. E., and Namerow, K. N. (2010) Dental pulp stem cell migration. J. Endod. 36, 1963–1966
12. Schwalm, S., Pleilshijfer, J., and Huwiler, A. (2010) Sphingosine kinase 1 is critically involved in nitric oxide-mediated human endothelial cell migration and tube formation. Br. J. Pharmacol. 160, 1641–1651
13. Pereira, J. P., Kelly, L. M., and Cyster, J. G. (2010) Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. Int. Immunol. 22, 413–419
14. Harvey, K. A., Welch, Z., Silva, D., and Siddiqui, R. A. (2010) Role of Rho kinase in sphingosine 1-phosphate-mediated endothelial and smooth muscle cell migration and differentiation. Mol. Cell. Biochem. 342, 7–19
15. Simonson, J. L., Rosada, C., Serakinci, N., Justesen, J., Stenderup, K., Rat- tan, S. I., Jensen, T. G., and Kassem, M. (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat. Biotechnol. 20, 592–596
16. Kratchmarova, I., Blagoev, B., Haack-Sorensen, M., Kassem, M., and Mann, M. (2005) Mechanism of divergent growth factor effects in mesenchymal stem cell differentiation. Science 308, 1472–1477
17. McVerry, B. J., and Garcia, J. G. (2005) In vitro and in vivo modulation of vascular barrier integrity by sphingosine 1-phosphate: mechanistic insights. Cell. Signal. 17, 131–139
18. Belvitch, P., and Dudek, S. M. (2012) Role of FAK in S1P-regulated endothelial permeability. Microvasc. Res. 83, 22–30
19. Wang, S., and Basson, M. D. (2011) Protein kinase B/AKT and focal adhesion kinase: two close signaling partners in cancer. Anticancer Agents Med. Chem. 11, 993–1002
20. Brown, S., Zeidler, M. P., and Hombria, J. E. (2006) IAK/STAT signalling in Drosophila controls cell motility during germ cell migration. Dev. Dyn. 235, 958–966
21. Yamada, O., Ozaki, K., Akiyama, M., and Kawachui, K. (2012) IAK-STAT and JAK-PI3K-mTORC1 pathways regulate telomerase transcriptionally and posttranslationally in ATL cells. Mol. Cancer Ther. 11, 1112–1121
22. Nagiec, M. M., Skrzypek, M., Nagiec, E. E., Lester, R. L., and Dickson, R. C. (1998) The LCB4 (YOR171c) and LCB5 (YLR266w) genes of Saccharomyces encode sphingoid long chain base kinases. J. Biol. Chem. 273, 19437–19442
23. Mizugishi, K., Yamashita, T., Serakinci, N., Justesen, J., Stenderup, K., Rat- tan, S. I., Jensen, T. G., and Kassem, M. (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat. Biotechnol. 20, 592–596
24. Wu, Y. P., Mizugishi, K., Bektas, M., Sandhoff, R., and Proia, R. L. (2008) Sphingosine kinase 1/2 and protein kinase C-alpha regulating ML-1 thyroid carcinoma cell migration. J. Biol. Chem. 283, 19437–19442
25. Muzigishi, K., Yamashita, T., Serakinci, N., Justesen, J., Stenderup, K., Rat- tan, S. I., Jensen, T. G., and Kassem, M. (2002) Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat. Biotechnol. 20, 592–596
28. See, Y. J., Alexander, S., and Hahn, B. (2011) Does cytokine signaling link sphingolipid metabolism to host defense and immunity against virus infections? Cytokine Growth Factor Rev. 22, 55–61.

29. Li, M. H., Sanchez, T., Yamase, H., Hla, T., Oo, M. L., Pappalardo, A., Lynch, K. R., Lin, C. Y., and Ferrer, F. (2009) S1P1/S1P1 signaling stimulates cell migration and invasion in Wilms tumor. Cancer Lett. 276, 171–179.

30. Zeng, H., Zhao, D., and Mukhopadhyay, D. (2002) KDR stimulates endothelial cell migration through heterotrimeric G protein G11-mediated activation of a small GTPase RhoA. J. Biol. Chem. 277, 46791–46798.

31. Soede, R. D., Zeelenberg, I. S., Wijnands, Y. M., Kamp, M., and Roos, E. (2001) Stromal cell-derived factor-1-induced LFA-1 activation during in vivo migration of T cell hybridoma cells requires G11/RhoA, and myosin, as well as G, and Cdc42. J. Immunol. 166, 4293–4301.

32. O’Connor, K. L., Nguyen, B. K., and Mercurio, A. M. (2000) RhoA function in lamellae formation and migration is regulated by the α6β4 integrin and cAMP metabolism. J. Cell Biol. 148, 253–258.

33. Matsuysuki, H., Maeda, Y., Yan, K., Sugahara, K., Chiba, K., Kohn, T., and Igasaki, Y. (2006) Involvement of sphingosine-1-phosphate (S1P) receptor type 1 and type 4 in migratory response of mouse T cells toward S1P. Cell. Mol. Immunol. 3, 429–437.

34. Stambolic, V., and Woodgett, J. R. (2006) Functional distinctions of protein kinase B/Akt isoforms defined by their influence on cell migration. Trends Cell Biol. 16, 461–466.

35. Delehedde, M., Sergeant, N., Lyon, M., Rudland, P. S., and Fernig, D. G. (2001) Hepatocyte growth factor/scatter factor stimulates migration of rat mammary fibroblasts through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt pathways. Eur. J. Biochem. 268, 4423–4429.

36. Bonacchi, A., Romagnani, P., Romanelli, R. G., Effen, E., Annunziato, F., Lasagni, L., Francalanci, M., Serio, M., Laffi, G., Pinzani, M., Gentilini, P., and Marra, F. (2001) Signal transduction by the chemokine receptor CXCR3: activation of Ras/ERK, Src, and phosphatidylinositol 3-kinase/Akt pathways. J. Biol. Chem. 276, 9945–9954.

37. Zheng, D. Q., Woodard, A. S., Tallini, G., and Languino, L. R. (2000) Substrate specificity of α5β3 integrin-mediated cell migration and phosphatidylinositol 3-kinase/Akt pathway activation. J. Biol. Chem. 275, 24565–24574.

38. Morales-Ruiz, M., Fulton, D., Sowa, G., Languino, L. R., Fujio, Y., Walsh, K., and Sessa, W. C. (2000) Vascular endothelial growth factor-stimulated actin reorganization and migration of endothelial cells is regulated via the serine/threonine kinase Akt. Circ. Res. 86, 892–896.

39. Badgwell, D. B., Lu, Z., Le, K., Gao, F., Yang, M., Suh, G. K., Bao, J. J., Das, P., Andreuff, M., Chen, W., Yu, Y., Ahmed, A. A., Liao, W., and Bast, R. C., Jr. (2012) The tumor-suppressor gene ARHI (DIRAS3) suppresses ovarian cancer cell migration and invasion, and angiogenesis in multiple tumor lineages. Cancer Cell 9, 225–238.

40. Adler, I., Malavaud, B., and Cuvillier, O. (2009) When the sphingosine kinase 1/sphingosine-1-phosphate pathway meets hypoxia signaling: new targets for cancer therapy. Cancer Res. 69, 3723–3726.

41. Cuvillier, O. (2007) Sphingosine kinase-1: a potential therapeutic target in cancer. Anticancer Drugs 18, 105–110.

42. Johnson, C. E., Gorringe, K. L., Thomson, E. R., Opeskin, K., Boyle, S. E., Wang, Y., Hill, P., Mann, G. B., and Campbell, I. G. (2012) Identification of copy number alterations associated with the progression of DCIS to invasive ductal carcinoma. Breast Cancer Res. Treat. 133, 889–898.

43. Guan, H., Liu, L., Cai, J., Liu, J., Ye, C., Li, M., and Li, Y. (2011) Sphingosine kinase 1 is overexpressed and promotes proliferation in human thyroid cancer. Mol. Endocrinol. 25, 1858–1866.

44. Facchinetti, M. M., Gandini, N. A., Fermento, M. E., Sterin-Speziale, N. B., Ji, Y., Patel, V., Gurtik, J. S., Ravdulla, M. G., and Curino, A. C. (2010) The expression of sphingosine kinase-1 in head and neck carcinoma. Cells Tissues Organs 192, 314–324.

45. Kapitonen, D., Allegood, J. C., Mitchell, C., Hait, N. C., Almenara, J. A., Adams, J. K., Zipkin, R. E., Dent, P., Kordula, T., Milstien, S., and Spiegel, S. (2009) Targeting sphingosine kinase 1 inhibits Akt signaling, induces apoptosis, and suppresses growth of human glioblastoma cells and xenografts. Cancer Res. 69, 6915–6923.

46. Paugh, S. W., Paugh, B. S., Rahmani, M., Kapitonen, D., Almenara, J. A., Kordula, T., Milstien, S., Adams, J. K., Zipkin, R. E., Grant, S., and Spiegel, S. (2008) A selective sphingosine kinase 1 inhibitor integrates multiple molecular therapeutic targets in human leukemia. Blood 112, 1382–1391.

47. Lai, W. Q., Irwan, A. W., Goh, H. H., Howe, H. S., Yu, D. T., Valle-Oñate, R., McInnes, I. B., Melendez, A. J., and Leung, B. P. (2008) Anti-inflammatory effects of sphingosine kinase modulation in inflammatory arthritis. J. Immunol. 181, 8010–8017.

48. Maires, L. W., Fitzpatrick, L. R., French, K. J., Zhuang, Y., Xia, Z., Keller, S. N., Upson, J. I., and Smith, C. D. (2008) Suppression of ulcerative colitis in mice by orally available inhibitors of sphingosine kinase. Dig. Dis. Sci. 53, 997–1012.

49. French, K. J., Upson, J. I., Keller, S. N., Zhuang, Y., Yun, J. K., and Smith, C. D. (2006) Antitumor activity of sphingosine kinase inhibitors. J. Pharmacol. Exp. Ther. 318, 596–603.

50. Lai, W. Q., Chia, F. L., and Leung, B. P. (2012) Sphingosine kinase and sphingosine-1-phosphate receptors: novel therapeutic targets of rheumatoid arthritis? Future Med. Chem. 4, 727–733.

51. Wadgaonkar, R., Patel, V., Grinkina, N., Romano, C., Liu, J., Zhao, Y., Sammani, S., Garcia, J. G., and Natarajan, V. (2009) Differential regulation of sphingosine kinases 1 and 2 in lung injury. Am. J. Physiol. Lung Cell Mol. Physiol. 296, L603–L613.

52. Jin, Z. Q., Zhang, J., Huang, Y., Hoover, H. E., Vessey, D. A., and Karliner, J. S. (2007) A sphingosine kinase 1 mutation sensitizes the myocardium to ischemia/reperfusion injury. Cardiaco. Res. 67, 41–50.

53. Hla, T., Venkataraman, K., and Michaud, I. (2008) The vascular S1P gradient—cellular sources and biological significance. Biochim. Biophys. Acta 1781, 477–482.

54. Takabe, K., Paugh, S. W., Milstien, S., and Spiegel, S. (2008) “Inside-out” signaling of sphingosine-1-phosphate: therapeutic targets. Pharmacol. Rev. 60, 181–195.

55. Oskeritzian, C. A., Alvarez, S. E., Hait, N. C., Price, M. M., Milstien, S., and Spiegel, S. (2008) Distinct roles of sphingosine kinases 1 and 2 in human mast-cell functions. Blood 111, 4193–4200.

56. Billich, A., Bornancin, F., Mechtcheriakova, D., Natt, F., Huesken, D., and
Baumruker, T. (2005) Basal and induced sphingosine kinase 1 activity in A549 carcinoma cells: function in cell survival and IL-1β and TNF-α induced production of inflammatory mediators. Cell. Signal. 17, 1203–1217

65. Wu, W., Mosteller, R. D., and Broek, D. (2004) Sphingosine kinase protects lipopolysaccharide-activated macrophages from apoptosis. Mol. Cell. Biol. 24, 7359–7369

66. Pettus, B. J., Bielawski, J., Porcelli, A. M., Reames, D. L., Johnson, K. R., Morrow, J., Chalfant, C. E., Obeid, L. M., and Hannun, Y. A. (2003) The sphingosine kinase 1/sphingosine 1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF-α. FASEB J. 17, 1411–1421