Akt1/Akt2 and Mammalian Target of Rapamycin/Bim Play Critical Roles in Osteoclast Differentiation and Survival, Respectively, Whereas Akt Is Dispensable for Cell Survival in Isolated Osteoclast Precursors*

Received for publication, September 13, 2004, and in revised form, November 4, 2004
Published, JBC Papers in Press, November 15, 2004, DOI 10.1074/jbc.M410480200

Toshifumi Sugatani and Keith A. Hruska‡
From the Department of Pediatrics, Cell and Molecular Biology Unit, Washington University School of Medicine, St. Louis, Missouri 63110

Akt, also known as protein kinase B, is a serine/threonine protein kinase with antiapoptotic activities; also, it is a downstream target of phosphatidylinositol 3-kinase. Here we show that Akt1/Akt2 play a critical role in osteoclast differentiation but not cell survival and that mammalian target of rapamycin (mTOR) and Bim, a pro-apoptotic Bcl-2 family member, are required for cell survival in isolated osteoclast precursors. To investigate the function of Akt1, Akt2, mTOR, and Bim, we employed a retroviral system for delivery of small interfering RNA into cells. Loss of Akt1 and/or Akt2 protein inhibited osteoclast differentiation due to down-regulation of IκB kinase (IKK) α/β activity, phosphorylation of IκB-α, nuclear translocation of nuclear factor-κB (NFκB) p50, and NFκB p50 DNA-binding activity. Surprisingly, deletion of Akt1 and/or Akt2 protein did not stimulate cleaved caspase-3 activity and failed to promote apoptosis. Conversely, loss of mTOR protein induced apoptosis due to up-regulation of cleaved caspase-3 activity. In addition, we found that mTOR is downstream of phosphatidylinositol 3-kinase (but not Akt) and that macrophage colony-stimulating factor regulates Bim expression through mTOR activation for cell survival. These results demonstrate that Akt1/Akt2 are key elements in osteoclast differentiation and that the macrophage colony-stimulating factor stimulation of mTOR leading to Bim inhibition is essential for cell survival in isolated osteoclast precursors.

Akt is a downstream effector of phosphatidylinositol 3-kinase (PI3K) and has been shown to be a critical mediator of cell proliferation and survival in a variety of cell types (1). There are three Akt genes, which yield Akt1, Akt2, and Akt3 (2). All three gene products are similar in structure and size, contain an amino-terminal pleckstrin homology domain and seem to be regulated by similar mechanisms (2). Akt1 is the most ubiquitously expressed isoform in mammalian cells and tissues (2). Akt2 is also expressed in most tissues and organs, usually at levels lower than Akt1 (2). Akt3 is expressed at the lowest level in most adult tissues, except testes and brain (2).

Recently, Akt has been implicated in cell differentiation (3, 4). For example, several studies have reported that both mRNA and protein levels of endogenous Akt1 are not changed, whereas Akt2 is elevated during muscle differentiation, suggesting that Akt2, but not Akt1, plays an important role of myogenesis (3). Consistent with these results, Akt enhances MyoD transactivation, and MyoD promotes Akt expression during muscle differentiation (3). Peng et al. (5) have demonstrated that adipocyte differentiation is blocked in Akt1/Akt2 double knock-out (DKO) mice, and Akt is required for the induction of peroxisome proliferator-activated receptor γ expression (the master regulator of adipogenesis) during adipocyte differentiation. Akt1/Akt2 DKO mice also displayed delayed bone development (5). However, the function of Akt1/Akt2 during osteoclastogenesis is largely unknown.

NFκB is an essential transcription factor for osteoclast differentiation because there are no osteoclasts in NFκB p50/p52 DKO (6). In addition, it has been reported that NFκB is a target of Akt (7, 8). Moreover, IκB kinase α (IKKα), which is upstream of NFκB, has an Akt phosphorylation site (7). Therefore, we hypothesized that Akt regulates osteoclast differentiation and cell survival in isolated osteoclast precursors. To investigate our postulate, we employed a retroviral system for delivery of small interfering RNA (siRNA) into cells, and found that Akt1/Akt2 play a critical role of osteoclast differentiation but not cell survival. Furthermore, the macrophage colony-stimulating factor (M-CSF)-stimulated mammalian target of rapamycin (mTOR)/Bim signal is required for cell survival in osteoclast precursors.

**EXPERIMENTAL PROCEDURES**

**Materials**—Polyclonal anti-Akt, monoclonal anti-Akt1, polyclonal anti-Akt2, polyclonal anti-phospho-Akt (threonine 308), polyclonal anti-phospho-Akt (serine 473), polyclonal anti-mTOR, polyclonal anti-phospho-mTOR, polyclonal anti-IκBα, polyclonal anti-IκBβ, polyclonal anti-IκB-α, anti-polyclonal mouse IgG HRP-linked antibody, anti-rabbit IgG HRP-linked antibody, LY294002 (inhibitor of PI3K), and rapamycin (inhibitor of mTOR) were purchased from Cell Signaling. Technology. Polyclonal anti-IκB kinase, which is upstream of NFκB, has an Akt phosphorylation site (7). Therefore, we hypothesized that Akt regulates osteoclast differentiation and cell survival in isolated osteoclast precursors. To investigate our postulate, we employed a retroviral system for delivery of small interfering RNA (siRNA) into cells, and found that Akt1/Akt2 play a critical role of osteoclast differentiation but not cell survival. Furthermore, the macrophage colony-stimulating factor (M-CSF)-stimulated mammalian target of rapamycin (mTOR)/Bim signal is required for cell survival in osteoclast precursors.

**This paper is available on line at http://www.jbc.org**
mouse siRNA target sequences were designed by Dharmacon (Table I). The 56-mer sense and antisense strands of DNA oligonucleotides were synthesized by Integrated DNA Technologies. Construction of a retroviral vector for delivery of siRNA and generation of virus were performed using the GeneSuppressor retroviral system (Imgenex) according to the manufacturer’s suggestions.

**Cell Culture and Infection**—Mouse bone marrow macrophages (BMMs) were prepared from the femur and tibia of 4–6-week-old C57BL/6 mice and incubated in tissue culture dishes (100-mm dish) in the presence of recombinant mouse M-CSF (20 ng/ml). After 24 h in culture, the non-adherent cells were collected and layered on Histopaque gradient (Sigma), and the cells at the gradient interface were collected. The cells were replated (60-mm dish) in α minimum Eagle’s medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (Biocol Laboratories) in the presence of M-CSF (100 ng/ml). After 3 days in culture, the cells were infected with siRNA retrovirus for 24 h in the presence of 100 ng/ml M-CSF and 4 μg/ml polybrene (Sigma). Infected cells were cultured for an additional 2–3 days, and the cells were harvested for assay. Further, for *in vitro* differentiation, 3.2 × 10^5 cells/well were plated in 24-well plates in the presence of RANKL (100 ng/ml) and M-CSF (20 ng/ml). After 4 days, the cells were stained for tartrate-resistant acid phosphatase (TRAP) activity (Sigma). TRAP-positive multinucleated cells containing more than three nuclei were counted as osteoclasts under microscopic examination.

**Western Blotting**—For LY294002 (50 μM), rapamycin (1 ng/ml), Akt1⁄Akt2, NF-κB p50, and Bim, the cells were then washed once with ice-cold phosphate-buffered saline and lysed in a cell lysis buffer (New England Biolabs) to prepare whole-cell lysates, and the lysates were confirmed by centrifugation at 14,000 × g for 10 min. For the detection of NF-κB p50, nuclear extracts were employed, instead of whole-cell lysates, using the nuclear and cytoplasmic extraction reagent (Pierce) according to the manufacturer’s suggestions. Protein concentrations of whole-cell lysates and nuclear extraction were measured using the Bio-Rad protein assay reagent (Bio-Rad). Proteins were resolved by SDS-PAGE, electroblotted to polyvinylidene difluoride membrane (Millipore), blocked in 10% skim milk, 1% BSA, reprobed in 0.05% Tween 20, and probed with primary antibodies. Following incubation with anti-mouse IgG HRP-linked antibody or anti-rabbit IgG HRP-linked antibody, bound immunoglobulins were detected using enhanced chemiluminescence (Pierce).

**NF-κB p50 DNA-binding Activity**—After treatment with RANKL (100 ng/ml) for the indicated time, the infected cells (72 h post-infection) were harvested. Afterward, nuclear extraction was prepared as described previously. NF-κB p50 DNA-binding activity of infected cells by RANKL was measured using the Mercury Transfactor kit (BD Biosciences) according to the manufacturer’s instructions.

**Cleaved Caspase-3 Activity**—After 2–3 days of infection, retrovirally transduced cells were harvested. Thereafter, whole-cell lysates were prepared as described previously. Lysates were confirmed by centrifugation at 14,000 × g for 10 min, and the supernatant fraction was harvested. Cleaved caspase-3 activity assay of cell extracts was measured using the PathScan cleaved caspase-3 sandwich enzyme-linked immunosorbent assay kit (Cell Signaling) according to the manufacturer’s suggestions.

**Apoptosis in Situ Marker**—Infected cells undergoing apoptosis were identified by the CaspACE fluorescein isothiocyanate-VAD-fluoromethyl ketone *in situ* marker (Promega). A fluorescein isothiocyanate conjugate of the cell permeable caspase inhibitor VAD-fluoromethyl ketone strongly labeled activated caspase, serving as an *in situ* marker for apoptosis. The bound marker was localized by fluorescence detection.

**Cell Viability Assay**—To deplete M-CSF, infected cells were washed twice with α minimum Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum in the absence of M-CSF after 3 days of infection. The infected cells were further cultured for the indicated times, and then viable cell counts were determined by trypan blue dye exclusion (Sigma) in triplicate assays. The number of viable infected cells remaining at the different time points is shown as a percentage of the infected cells at time zero.

**RESULTS AND DISCUSSION**

**siRNA-induced Gene Silencing in Isolated Osteoclast Precursors**—To test whether Akt1, Akt2, mTOR, and Bim siRNA could induce gene-specific silencing in primary bone marrow macrophages, we first performed Western blotting to measure these expressed proteins. The loss of Akt1 inhibited the expression of Akt1 protein at both 48 and 72 h after infection, whereas Akt2 protein expression was unaffected (Fig. 1A). Similarly, depletion of Akt2 suppressed the expression of Akt2 protein; however, Akt1 protein expression had no effects (Fig. 1B). In addition, mTOR and Bim siRNA gene silencing also abolished these protein expressions by 48 and 72 h post-infection (Fig. 1, C and D).

**Akt1/Akt2 Are Essential for Osteoclast Differentiation**—Despite histopathological examination demonstrating no significant differences between wild-type and Akt1-deficient mice in most adult tissues, except testes, Akt1-deficient mice were smaller when compared with wild-type littersmates (10). Similarly, Akt2-null mice also were smaller than wild-type mice (11). However, these reports do not describe the influence of Akt on bone tissues. In contrast, Akt1/Akt2 DKO revealed delay of bone development and small bodies compared with control mice (5). However, chondrogenesis, osteoblastogenesis, and osteoclastogenesis in Akt1/Akt2...
Akt2 DKO are largely unknown. Thus, Akt seems to play an important role in skeletal development.

To analyze the role of Akt in osteoclastogenesis, we utilized RANKL-induced differentiation of osteoclast precursors. RANKL activates the IKKα/β/NFκB axis for osteoclastogenesis (6). Ordinarily, NFκB is sequestered in the cytoplasm by the inhibitory protein IκBα. RANKL promotes IκB degradation, thereby allowing NFκB to enter the nucleus and induce gene transcription involved in osteoclast differentiation (6). We investigated whether loss of Akt1 and/or Akt2 protein suppresses osteoclast differentiation. As predicted, Western blot analysis using an antibody specific for the phosphorylated form of IκKα/β and IκB-α showed that IκKα/β and IκB-α were transiently and rapidly phosphorylated by 5 min after RANKL stimulation in scrambled siRNA expressing cells, and nuclear translocation of NFκB p50 was induced by RANKL at 5 min in control cells (Fig. 2). However, these effects of RANKL were inhibited by Akt1 and/or Akt2 siRNA gene silencing (Fig. 2).

A

RANKL

IB: p-IKKα/β
Scrambled siRNA

IB: IκKα
Akt1 siRNA

IB: p-IKKα/β
Akt2 siRNA

IB: IκKα
Akt1+2 siRNA

B

RANKL

IB: p-IκB-α
Scrambled siRNA

IB: IκB-α
Akt1 siRNA

IB: p-IκB-α
Akt2 siRNA

IB: IκB-α
Akt1+2 siRNA

C

RANKL

IB: NFκB p50
Scrambled siRNA

Akt1 siRNA

Akt2 siRNA

Akt1+2 siRNA

Fig. 2. Akt1 and/or Akt2 gene silencing suppresses the RANKL-induced IKK/IκB-NFκB signal. A–C, after infection for 72 h, BMMs were treated with RANKL (100 ng/ml) for the indicated times, and whole-cell extracts (A and B) or nuclear extracts (C) were analyzed by immunoblotting (IB).

Akt1 and/or Akt2 are essential for osteoclast differentiation. 72 h post-infection, BMMS were replaced in 24-well plates, and the cells were treated with RANKL (100 ng/ml) and M-CSF (20 ng/ml). After 4 days, the cells were then fixed and stained for TRAP, and the number of TRAP-positive multinucleated cells (MNCs) was scored. Similar findings were obtained in four independent sets of experiments. *, p < 0.01 compared with scrambled siRNA-expressing cells.

Fig. 4. Akt1/Akt2 are essential for osteoclast differentiation. Akt1/Akt2 are essential for osteoclastogenesis but not survival.
Akt1/Akt2, Essential for Osteoclastogenesis but Not Survival

indicated times (Fig. 3). Consistent with these results, the TRAP-positive osteoclast formation number was reduced in Akt1 and/or Akt2 siRNA-expressing cells with RANKL and M-CSF (Fig. 4), indicating that Akt1/Akt2 play an essential role in osteoclast differentiation.

Akt1/Akt2 Are Dispensable for Cell Survival of Isolated Osteoclast Precursors—Akt1 and/or Akt2 siRNA gene silencing suppressed TRAP-positive osteoclast formation (Fig. 4). However, the possibility remains that the TRAP-positive osteoclast formation number was further reduced by Akt1 and/or Akt2 siRNA-induced apoptosis. To explore this possibility, we utilized a cleaved caspase-3 activity assay and apoptosis detection with an in situ fluorescent marker assay under treatment with M-CSF. The increase of cleaved caspase-3 activity is observed in the cells undergoing apoptosis (12). Surprisingly, loss of Akt1 and/or Akt2 protein failed to induce cleaved caspase-3 activity by 48 and 72 h post-infection (Fig. 5A). Apoptotic cells also were not detected in Akt1 and/or Akt2 siRNA-expressing cells by fluorescence microscopy at 72 h post-infection (Fig. 5B). Moreover, we performed cell viability assays using trypan blue staining to measure cell survival rate in Akt1 and Akt2 siRNA-expressing cells. However, there was no difference between control cells and infected cells in the indicated times after M-CSF withdrawal (Fig. 5C). On the other hand, in these assays, Akt1 and/or Akt2 siRNA gene silencing induced apoptosis in the NIH-3T3 fibroblastic cell line (data not shown), strongly suggesting that Akt1/Akt2 are dispensable for cell survival in isolated osteoclast precursors.

mTOR and Bim Are Required for Cell Survival of Isolated Osteoclast Precursors—M-CSF is essential for survival and proliferation of osteoclast precursors (6). It was first reported that Akt plays the major role in the regulation of apoptosis in neuronal cells (13). After that, numerous studies have reported that Akt plays a central role of cell survival in most cells in vitro experiments (1, 2). However, Akt1-deficient mice displayed no significant increase in apoptosis in all other examined tissues, except testes and thymus, in TdT-mediated dUTP nick-end labeling (TUNEL) assays (10). Moreover, no increase in spontaneous apoptosis was observed in Akt1/Akt2 DKO newborns and embryonic day 18.5 embryos by TUNEL analysis (5). In skeletal biology, there is widespread belief that Akt is essential for the cell survival of isolated mature osteoclasts (6, 14, 15). However, so far, no data of Akt cell survival function on M-CSF signaling in isolated osteoclast precursors and osteoclasts lacking Akt1 and/or Akt2 have been published. Consistent with our data, recent studies have shown that Akt is not required for cell survival in hematopoietic cells (16–18). The cells of the osteoclast lineage also develop from hematopoietic cells of the monocyte-macrophage lineage (6). What does regulate cell survival and apoptosis of osteoclast precursors? The first candidate is Bad. However, phosphorylation of Bad, which occurs downstream of Akt, does not play an important role in the cytokine-mediated survival of hematopoietic cells (16–18). We also failed to detect Bad phosphorylation by RANKL and M-CSF in isolated osteoclast precursors (19). The second candidate is NFκB, because NFκB inhibitors induce apoptosis of isolated mature osteoclasts and inhibit bone resorption (20).
However, no increase in apoptosis has been observed in cells of the osteoclast lineage in NFκB p50/p52 DKO (21). The third candidate is mTOR. mTOR is a key downstream mediator of hematopoietic cell survival (22). Previously, we have demonstrated, using rapamycin (an inhibitor of mTOR), that mTOR is essential for the survival of isolated osteoclast precursors, and rapamycin completely blocked osteoclast formation (19). Moreover, M-CSF, TNF-α, and RANKL also promote osteoclast survival by signaling through mTOR (23). The final candidate is Bim, a member of the BH3-only subfamily of cell death activators (24). Bim down-regulation has important implications for cell survival in hematopoietic cells (24). Bim knock-out mice have shown that Bim is essential for apoptosis of T lymphocyte, B lymphocyte, and myeloid cells (24). Moreover, Bim knock-out mice exhibited mild osteosclerosis (24). The life span of Bim−/− osteoclasts were elongated and resistant to cytokine withdrawal-induced apoptosis, but osteoclasts (Bim−/−) had abnormally low bone resorption activity (24).

We investigated whether loss of mTOR promoted apoptosis in isolated osteoclast precursors. Depletion of mTOR protein markedly induced cleaved caspase-3 activity compared with control after infection at 48 and 72 h (Fig. 5A). Moreover, apoptotic cells were obviously observed in mTOR siRNA-expressing cells by 72 h post-infection using the detection of apoptosis by in situ marker assay (Fig. 5B). In contrast, Bim siRNA gene silencing, expected to increase survival, failed to stimulate cleaved caspase-3 activity, and we were not able to detect apoptotic cells in Bim siRNA-expressing cells (Fig. 5, A and B). In addition, in the cell viability assay, mTOR siRNA gene silencing quickly induced apoptosis by 3 h after M-CSF withdrawal, and most cells died by 12 h (Fig. 5C). In contrast, loss of Bim protein induced resistance to M-CSF withdrawal-induced apoptosis by 12 and 24 h compared with control (Fig. 5C). Moreover, depletion of mTOR protein completely blocked osteoclast formation; however, it seems that this effect is the influence of apoptosis by mTOR siRNA gene silencing (Fig. 4). These results indicate that M-CSF-mediated mTOR and Bim are essential for cell survival in osteoclast precursors.

Akt1/Akt2, Essential for Osteoclastogenesis but Not Survival

Akt1/Akt2 Are Dispensable for Cell Proliferation—Akt and mTOR regulate cell proliferation in most cells (25). Although M-CSF stimulated cell proliferation in scrambled, Akt1, and/or Akt2 siRNA-expressing cells, depletion of Akt1 or Akt2 protein suppressed cell proliferation when compared with control by 72 and 96 h post-infection. Moreover, loss of Akt1 and Akt2 protein also inhibited cell proliferation when compared with scrambled, Akt1, or Akt2 siRNA-expressing cells by 72 and 96 h after infection (Fig. 6). In contrast, consistent with previous reports, mTOR siRNA gene silencing failed to induce cell proliferation.
proliferation (Fig. 6), indicating that Akt1/Akt2 do not play a critical role of cell proliferation in osteoclast precursors.

mTOR Down-regulates Bim Expression for Cell Survival in Isolated Osteoclast Precursors—Both mTOR and Akt1/Akt2 are downstream effectors of the PI3K/Akt signaling pathway, which mediates cell survival in the most cells (25). However, we have shown that Akt1/Akt2 are not required for cell survival in osteoclast precursors (Fig. 5). Thereby, we postulated that the activation of mTOR is positively regulated by PI3K (but not by Akt) and that mTOR regulates Bim expression for cell survival in osteoclast precursors under treatment with M-CSF. To study these hypotheses, we used LY294002 (inhibitor of PI3K), rapamycin, (inhibitor of mTOR) and carried out Akt1, Akt2, or mTOR siRNA retrovirus infection into isolated osteoclast precursors (Fig. 5). Therefore, we postulated that the activation of mTOR is positively regulated by PI3K (but not by Akt) and that mTOR regulates Bim expression for cell survival in osteoclast precursors under treatment with M-CSF. To study these hypotheses, we used LY294002 (inhibitor of PI3K), rapamycin, (inhibitor of mTOR) and carried out Akt1, Akt2, or mTOR siRNA retrovirus infection into isolated osteoclast precursors. Although M-CSF activated Akt (threonine 308 and serine 473) and mTOR by 5 min in osteoclast precursors, these effects were blocked by LY294002 (Fig. 7, A and B). Moreover, M-CSF phosphorylated mTOR by 5 min in scrambled, Akt1, or Akt2 siRNA-expressing cells. In contrast, rapamycin blocked phosphorylation of mTOR by M-CSF; however, rapamycin failed to block phosphorylation of Akt (threonine 308 and serine 473) in the indicated times (Fig. 7, C and D), suggesting that mTOR is a downstream effector of the PI3K (but not Akt) in isolated osteoclast precursors. In addition, the expression of Bim gradually increased by 3 h after M-CSF withdrawal in control cells, whereas M-CSF completely blocked Bim expression (Fig. 8A). In contrast, Bim expression continued at high levels in mTOR siRNA-expressing cells without M-CSF in the indicated times, and M-CSF failed to affect Bim expression (Fig. 8B). This indicates that Bim is downstream of mTOR, and M-CSF-mediated mTOR down-regulates Bim expression for cell survival in isolated osteoclast precursors. Down-regulation of Bim by siRNA increased osteoclast precursor survival following M-CSF withdrawal (Fig. 5C) and in mTOR siRNA expressing cells (not shown).

As shown in Fig. 9, our results implicate activation of Akt1/Akt2 as a key event in osteoclast differentiation mediated through NFκB but not cell survival. The AP-1 component c-fos is also a very important transcription factor, because mice without this protein lack osteoclasts and are osteopetrotic but have an increased number of bone marrow macrophages (6). In this study, we have demonstrated that Akt1/Akt2 regulate NFκB p50 DNA-binding activity for osteoclast differentiation (Fig. 3), but Akt1 and/or Akt2 siRNA gene silencing failed to influence c-fos DNA-binding activity in isolated osteoclast precursors (data not shown). We also indicated that the mTOR/Bim signal is essential for cell survival of isolated osteoclast precursors (Figs. 5, 7, and 8). Surprisingly, Akt was dispensable for cell survival in our data (Fig. 5). This was an unexpected result for us. However, it has been shown that the PI3K/Akt signal can protect against apoptosis in hematopoietic cells (26, 27), whereas the role of Akt in this system has been controversial. Although several laboratories emphasized a marginal capacity of Akt to reverse apoptosis due to cytokine withdrawal (28, 29), accumulating evidence refutes a contribution of Akt to cell survival (16–18). In this study, our results support previous negative findings. Thus, we provide the first evidence that Akt activity is critical for osteoclast differentiation but not cell survival, and mTOR/Bim play central roles in cell survival of isolated osteoclast precursors.

Acknowledgments—We thank Dr. Toshio Doi and Dr. Kameswaran Sureshar for helpful discussions and Helen Odle for expert secretarial assistance.

REFERENCES

1. Scheid, M. P., and Woodgett, J. R. (2001) Nat. Rev. Mol. Cell. Biol. 2, 760–766
2. Datta, S. R., Brunet, A., and Greenberg, M. E. (1999) Genes Dev. 13, 2905–2927
3. Kaneko, S., Feldman, R. I., Yu, L., Wu, Z., Gritsko, T., Shelley, S. A., Nicosia, S. V., Nobori, T., and Cheng, J. Q. (2002) J. Biol. Chem. 277, 23253–23255
4. Minerdith, J. E., Fazeli, B., and Schwartz, M. A. (1993) Mol. Biol. Cell 4, 953–961
5. Peng, X-d., Xu, P. Z., Chen, M. L., Hahn-Windgassen, A., Sleen, J., Jacobs, J., Sundararajan, D., Chen, W. S., Crawford, S. E., Coleman, K. G., and Hay, N. (2003) Genes Dev. 17, 1352–1365
6. Boyle, W. J., Simonet, W. S., and Lacey, D. L. (2003) Nature 423, 337–342
7. Ozes, O. N., Mayo, L. D., Gustin, J. A., Pfeffer, S. R., Pfeffer, L. M., and Donner, D. B. (1999) Nature 401, 82–85
8. Romashkovka, J. A., and Makarov, S. S. (1999) Nature 401, 86–90
9. Jiang, Z. Y., Zhou, Q. L., Coleman, K. A., Chouinard, M., Boese, Q., and Czech, M. P. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 7569–7574
10. Chen, W. S., Xu, P. Z., Gottlob, K., Chen, M. L., Sokol, K., Shiyanova, T., Roninson, I., Weng, W., Suzuki, R., Tobe, K., Kadowaki, T., and Hay, N. (2001) Genes Dev. 15, 2203–2208
11. Garofalo, R. S., Orena, S. J., Rafidi, K., Torchia, A. J., Stock, J. L., Hildebrandt, J. G., Fazeli, B., and Schwartz, M. A. (1993) Mol. Biol. Cell 4, 953–961
12. Sugatani, T., Alvarez, U., and Hruska, K. A. (2003) J. Biol. Chem. 278, 5001–5008
13. 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
14. Wong, B. R., Besser, D., Kim, N., Arron, J. R., Volodogskaid, M., Hanafusa, H., and Choi, Y. (1999) Mol. Cell 4, 1041–1049
15. Lee, Z. H., and Kim, H. H. (2003) Biochem. Biophys. Res. Commun. 305, 211–214
16. Scheid, M. P., and Duronio, V. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7439–7444
17. Tan, H., Jacobs-Helber, S. M., Lawson, A. E., Penta, K., Wickrema, A., and Sawyer, S. T. (1999) Blood 93, 3757–3773
18. Hinton, H. J., and Welham, M. J. (1999) J. Immunol. 162, 7002–7009
19. Sugatani, T., Alvarez, U. M., and Hruska, K. A. (2003) J. Cell. Biochem. 90, 59–67
20. Ozaki, K., Takeda, H., Ishikari, H., Kitano, S., and Hanafusa, H. (1997) J. Biol. Chem. 272, 297–300
21. King, L., Bushnell, T. P., Careless, L., Tai, Z., Tondraivi, M., Siebenlist, U., Young, P., and Boyle, B. F. (2002) J. Bone Miner. Res. 17, 1200–1210
22. Hosoi, H., Dilling, M. B., Shikata, T., Liu, L. N., Shu, L., Ashmun, R. A., Germain, G. S., Abraham, R. T., and Houghton, P. J. (1991) Cancer Res. 51, 277–280
Akt1/Akt2, Essential for Osteoclastogenesis but Not Survival

23. Glantschnig, H., Fisher, J. E., Wesolowski, G., Rodan, G. A., and Reszka, A. A. (2003) Cell Death Differ. 10, 1165–1177
24. Akiyama, T., Bouillet, P., Miyazaki, T., Kadono, Y., Chikuda, H., Chung, U., Fukuda, A., Hikita, A., Seto, H., Okada, T., Inaba, T., Sanjay, A., Baron, R., Kawaguchi, H., Oda, H., Nakamura, K., Strasser, A., and Tanaka, S. (2003) EMBO J. 22, 6653–6664
25. Panwalkar, A., Verstovsek, S., and Giles, F. J. (2004) Cancer 100, 657–666
26. Kinoshita, T., Yokota, T., Arai, K., and Miyajima, A. (1995) EMBO J. 14, 266–275
27. Kinoshita, T., Shirouzu, M., Kamiya, A., Hashimoto, K., Yokoyama, S., and Miyajima, A. (1997) Oncogene 15, 619–627
28. Ahmed, N. N., Grimes, H. L., Bellacosa, A., Chan, T. O., and Tsichlis, P. N. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3627–3632
29. Songyang, Z., Baltimore, D., Cantley, L. C., Kaplan, D. R., and Franke, T. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 11345–11350
Akt1/Akt2 and Mammalian Target of Rapamycin/Bim Play Critical Roles in Osteoclast Differentiation and Survival, Respectively, Whereas Akt Is Dispensable for Cell Survival in Isolated Osteoclast Precursors

Toshifumi Sugatani and Keith A. Hruska

J. Biol. Chem. 2005, 280:3583-3589.
doi: 10.1074/jbc.M410480200 originally published online November 15, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M410480200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 29 references, 14 of which can be accessed free at http://www.jbc.org/content/280/5/3583.full.html#ref-list-1