7. Blount, K.F., Wang, J.X., Lim, J., Sudarsan, N., and Breaker, R.R. (2007). Nat. Chem. Biol. *3*, 44–49.

8. Blount, K., Puskarz, I., Penchovsky, R., and Breaker, R. (2006). RNA Biol. *3*, 77–81.

9. Mayer, G., and Famulok, M. (2006). ChemBioChem *7*, 602–604.

10. Jenne, A., Hartig, J.S., Piganeau, N., Tauer, A., Samarsky, D.A., Green, M.R., Davies, J., and Famulok, M. (2001). Nat. Biotechnol. *19*, 56–61.

11. Breaker, R.R. (2002). Curr. Opin. Biotechnol. *13*, 31–39.

12. Que-Gewirth, N.S., and Sullenger, B.A. (2007). Gene Ther. *14*, 283–291.

13. Fisher, T.S., Joshi, P., and Prasad, V.R. (2002). J. Virol. *76*, 4068–4072.

14. Shi, H., Fan, X., Sevilimedu, A., and Lis, J.T. (2007). Proc. Natl. Acad. Sci. USA *104*, 3742–3746.

15. Nimjee, S.M., Rusconi, C.P., and Sullenger, B.A. (2005). Annu. Rev. Med. *56*, 555–583.

Mending the Bones with Natural Products

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In this issue, Qiu and colleagues [1] demonstrate in vitro and in vivo activity of a triterpene glycoside from black cohosh. The isolated compound acts as a suppressor of osteoclastogenesis, targeting specifically RANKL, a member of the TNF superfamily.

Bone-related diseases, such as Paget's disease, osteoporosis, arthritis, or cancer metastases, affect millions of people worldwide. Bones are constantly remodeled through the synthesis of bone matrix by osteoblasts and the resorption of bone by osteoclasts. Osteoblasts and osteoclasts arise from distinct cell lineages and maturation processes—osteoclasts arise from mesenchymal stem cells while osteoclasts differentiate from hematopoietic monocyte/macrophage precursors [2]. One of the key factors mediating the process of osteoclast formation known as osteoclastogenesis is receptor activator of nuclear factor-kappaB (NF-κB) ligand (RANKL), a member of the tumor necrosis factor (TNF) family that has also been called osteoclast differentiation factor, TNF-related activation-induced cytokine, and osteoprotegerin (OPG) ligand [3].

RANKL, protein expressed on the surface of osteoblastic/stromal cells, is directly involved in the differentiation of monocyte macrophages into osteoclasts [4]. Mice with disruptions in the *RANKL* gene show a lack of osteoclasts, severe osteopetrosis, and defective tooth eruption, indicating that RANKL-induced osteoclastogenesis is mediated through the cell surface receptor RANK [5]. That RANK can mediate osteoclastogenesis was first demonstrated by Hsu and coworkers in 1999 [6]. Further gene-deletion analysis of RANK, RANKL, and TNF receptor-associated factor 6 (TRAF6) showed that these genes are positive regulators of osteoclastogenesis [7], whereas OPG, a decoy receptor for

Table 1. Current Drugs Used for Bone Loss Related Disorders

Drug	Chemical Class	Mechanism of Action	Side Effects
Alendronate/Risedronate/ Ibandronate/Zoledronic Acid	Bisphosphonates	Inhibits osteoclast	GI toxicity, weight loss, bone pain, low calcium levels
Estrogen	Sex steroid	Inhibits osteoclast development	Endometrial cancer, stroke
Raloxifene	Estrogen mimic	Inhibits osteoclast development	Leg cramps, hot flashes
Estren	Estrogen derivative	Inhibits osteoblast apoptosis	Breast cancer
Denosumab	RANKL antibody	Inhibits osteoclast development	Nausea, diarrhea, cramps
Calcitonin	Peptide hormone	Inhibits osteoclasts	Nausea, skin redness, diarrhea
Teriparatide	Peptide	Induces bone formation	Pain, headache, diarrhea
PTHrP	Osteotrophin	Induces bone formation	Nausea, weakness

GI, gastrointestinal; RANKL, receptor activator of NF-κB ligand; PTHrP, parathyroid hormone-related peptide.

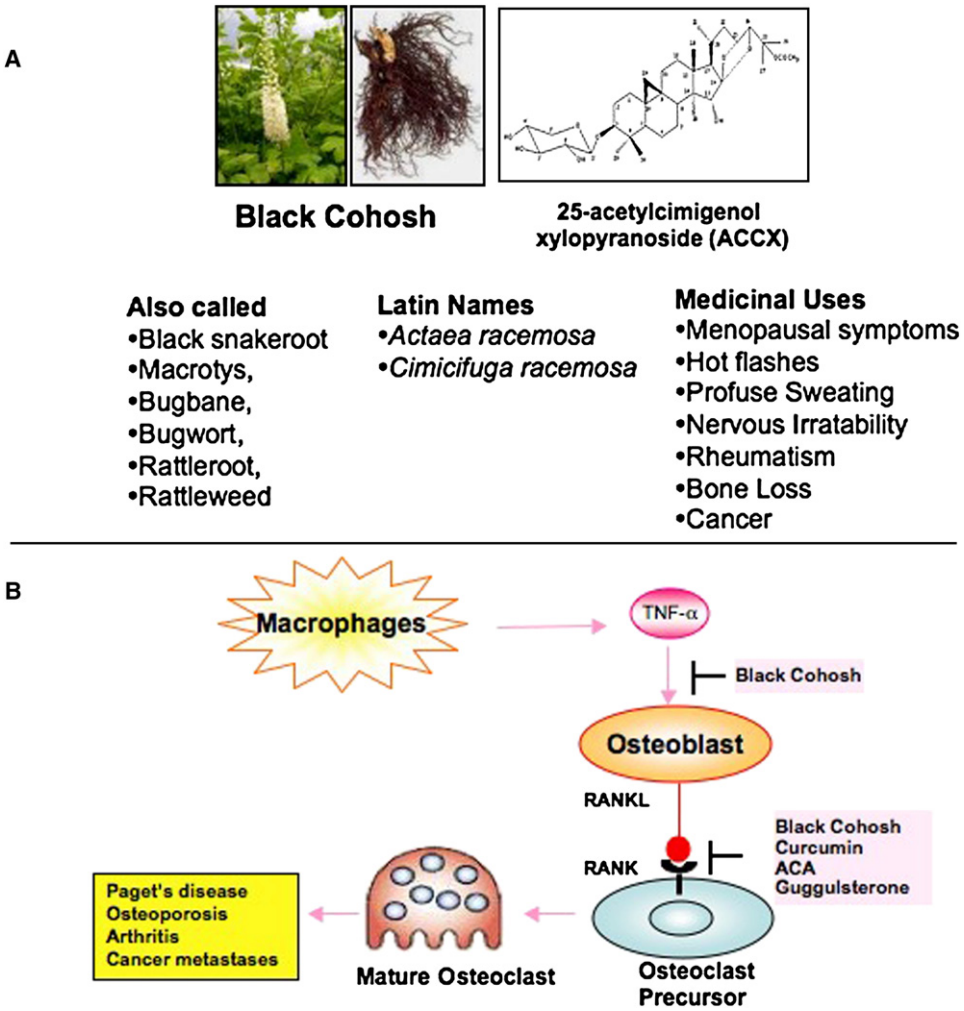

Figure 1. Role of Black Cohosh in Prevention of Bone Loss
(A) Black cohosh, its uses, and chemical structure of its active constituent ACCX.
(B) Suppression of osteoclastogenesis by natural agents.

RANKL, was found to be a negative regulator of this process [8]. RANKL-induced osteoclastogenesis is mediated through the cell surface receptor RANK. Through the recruitment of the adaptor proteins TRAF6 and NF-κB-inducing kinase (NIK), RANK activates transcription factor NF-κB and mitogen activated protein kinases (MAPK), such as c-Jun NH_2-terminal kinase (JNK), p38, and extracellular signal-regulated kinases (ERK1/2) signaling pathways [9]. Chronic inflammation is also an important mediator of osteoclastogenesis and a recent report indicates that people with the greatest number of inflammatory markers have the highest risk of osteoporotic fractures [10]. Current treatments for osteoporosis include estrogens and selective estrogen receptor modulators for suppressing osteoclast formation; and bis-phosphonates and calcitonin for suppressing osteoclast activity. However, most of these therapies have severe side effects, such as gastrointestinal toxicity and nausea (Table 1). Some new drug therapies for osteoporosis are on the horizon, such as an antibody against RANKL (Denosumab) that has been shown to increase bone mineral density and decrease bone resorption in postmenopausal women with low bone mass [11].

Because products from natural sources are safe and inexpensive, demonstration that they are also efficacious has a tremendous potential for the treatment of bone-related disorders. Several agents from natural sources such as curcumin (from turmeric), guggulsterone (from the guggul), and 1′-acetoxychavicol acetate (ACA; from Asian ginger) have been reported to suppress RANKL signaling and consequent osteoclastogenesis [12–14]. Most of these studies were done in cell cultures. Qiu and colleagues describe a compound from black cohosh that may have a potential against osteoporosis, based on their in vitro and in vivo studies [1]. Black cohosh, or *Actaea racemosa*, is a plant native to North America whose roots and rhizomes are widely used in the treatment of menopausal symptoms,

such as hot flashes, profuse sweating, insomnia, and anxiety (Figure 1A). This plant has also been shown to possess diuretic, antidiarrheal, and anti-inflammatory activity [15]. Qiu and colleagues showed that 25-acetylcimigenol xylopyranoside (ACCX), a cycloartane triterpenoid glycoside isolated from black cohosh, blocks osteoclastogenesis induced by either RANKL or TNF-α, with inhibition of the NF-κB and ERK signaling pathways [1]. Interestingly, they also demonstrated the efficacy of this compound on suppression of TNF-α-induced bone loss in vivo. They found that ACCX suppresses RANKL-induced activation of the NF-κB pathway as indicated by lack of phosphorylation and degradation of IκBα and inhibition of NF-κB DNA binding activity. They also observed that this triterpene downregulated RANKL-induced expression of NF-κB regulated genes, such as TNF-α- and toll-like receptor-2 (TLR2). RANKL-induced activation of MAPKs was also modulated by ACCX. They found that this compound induced inhibition of ERK activation whereas it activated p38 and JNK MAPKs.

This novel glycoside also inhibited TNF-α-induced osteoclastogenesis in vitro. This effect of ACCX was mediated through suppression of TNF-α-induced activation of NF-κB and ERK pathways. Most importantly, it was found that ACCX inhibited TNF-α-induced bone loss in vivo. These findings clearly establish that ACCX directly suppresses osteoporosis (Figure 1B), but it is not clear whether other components of black cohosh may also have a role in suppression of osteoclastogenesis. It is also not known whether ACCX can induce production of OPG in osteoblasts and therefore indirectly inhibit osteoclastogenesis.

The identification of RANKL, RANK, and OPG as key players in osteoclastogenesis has created new possibilities for designing highly effective and rational drugs to treat bone loss in millions of patients. Although targeting RANKL appears to be the most efficient and relevant approach for the treatment of bone loss, other strategies might be required to minimize potential side effects on other organs such as the innate and adaptive immune systems. Although numerous treatments for various bone-related disorders have been identified, they suffer from various drawbacks, such as lack of efficacy, excessive side effects, and high cost. Plant-derived products such as ACCX, curcumin, guggulsterone, ACA, and several others offer much promise for the treatment of diseases associated with bone loss but they require extensive investigation in various preclinical and clinical settings to prove their usefulness.

REFERENCES

1. Qiu, S.X., Dan, C., Ding, L.-S., Peng, S., Chen, S.-N., Farnsworth, N.R., Gross, M.L., and Zhou, P. (2007). Chem. Biol. *14*, this issue, 860–869.

2. Boyle, W.J., Simonet, W.S., and Lacey, D.L. (2003). Nature *423*, 337–342.

3. Aggarwal, B.B. (2003). Nat. Rev. Immunol. *3*, 745–756.

4. Teitelbaum, S.L. (2000). Science *289*, 1504–1508.

5. Kong, Y.Y., Yoshida, H., Sarosi, I., Tan, H.L., Timms, E., Capparelli, C., Morony, S., Oliveira-dos-Santos, A.J., Van, G., Itie, A., et al. (1999). Nature *397*, 315–323.

6. Hsu, H., Lacey, D.L., Dunstan, C.R., Solovyev, I., Colombero, A., Timms, E., Tan, H.L., Elliott, G., Kelley, M.J., Sarosi, I., et al. (1999). Proc. Natl. Acad. Sci. USA *96*, 3540–3545.

7. Wada, T., Nakashima, T., Hiroshi, N., and Penninger, J.M. (2006). Trends Mol. Med. *12*, 17–25.

8. Lacey, D.L., Timms, E., Tan, H.L., Kelley, M.J., Dunstan, C.R., Burgess, T., Elliott, R., Colombero, A., Elliott, G., Scully, S., et al. (1998). Cell *93*, 165–176.

9. Darnay, B.G., Haridas, V., Ni, J., Moore, P.A., and Aggarwal, B.B. (1998). J. Biol. Chem. *273*, 20551–20555.

10. Cauley, J.A., Danielson, M.E., Boudreau, R.M., Forrest, K.Y., Zmuda, J.M., Pahor, M., Tylavsky, F.A., Cummings, S.R., Harris, T.B., Newman, A.B., and for the Health ABC Study. (2007). J. Bone Miner. Res. *22*, 1088–1095.

11. Maricic, M. (2007). Curr. Opin. Rheumatol. *19*, 364–369.

12. Bharti, A.C., Takada, Y., and Aggarwal, B.B. (2004). J. Immunol. *172*, 5940–5947.

13. Ichikawa, H., and Aggarwal, B.B. (2006). Clin. Cancer Res. *12*, 662–668.

14. Ichikawa, H., Murakami, A., and Aggarwal, B.B. (2006). Mol. Cancer Res. *4*, 275–281.

15. Borrelli, F., Izzo, A.A., and Ernst, E. (2003). Life Sci. *73*, 1215–1229.

