BONE METASTASIS

Can osteoclasts be excluded?

Arising from: D. H. Jones *et al. Nature* 440, 692–696 (2006)

The RANK/RANKL signalling mechanism is the final common pathway of osteoclast formation and activity[1]. Inhibitors of RANK ligand (RANKL) that bind to RANK (for 'receptor activator of NF-κB'), such as osteoprotegerin (OPG), neutralizing antibodies against RANKL and soluble RANK antagonists, are well described inhibitors of bone metastasis in preclinical and clinical models, presumably because of their effects on osteoclasts[2]. Jones *et al.*[3] show that OPG inhibits bone metastasis after intracardiac injection of B16F10 murine melanoma cells, but claim that bone metastases are entirely independent of osteoclast formation and bone resorption: rather, they are caused by an effect on cell migration through RANK. However, we question whether these surprising conclusions are rigorously supported by their data[3].

The significance of RANK production by cancer cells has long been of interest, particularly as this TNF (for tumour-necrosis factor)-receptor family member is expressed in several breast-cancer cell lines and in a series of clinical breast cancers[4]. However, its role, if any, in the bone metastatic process is difficult to unravel in the absence of osteoclastic bone resorption. With this preclinical model of bone metastasis[5], tumour burden at bone metastatic sites is decreased whenever osteoclasts are inhibited — whether by bisphosphonates, inhibition of RANKL, neutralizing antibodies against parathyroid-hormone-related protein (PTHrP) or inhibitors of PTHrP transcription[2,6,7]. Evidence for a central role for osteoclasts in bone metastasis is therefore compelling.

Jones *et al.*[3] assume that osteoclasts have no role in their model of metastasis. To prove that, quantitative histology would be required, with the number of osteoclasts counted from the early stages of tumour-cell growth in bone. Once bone tumours have become large and generally invasive (which includes cell migration), it becomes difficult to see and quantify osteoclasts, even though classical large resorption-site Howship's lacunae are present. These lacunae are characteristic of osteoclasts and cannot be generated by any other cell, as shown in a scanning electron microscope study of human cancers metastasized to bone[8]. Jones *et al.*[3] indicate (their data are not shown) that treatment with the bisphosphonate zoledronic acid is ineffective in preventing bone-tumour growth. However, they do not provide the doses used, the timing of treatment, evidence that the zoledronic acid can inhibit osteoclasts, details of the behaviour of appropriate tumour controls (such as breast-cancer cells), or indication of whether treatment with OPG in the same experiment is effective.

Investigations into the role of the bone microenvironment in helping cancers to establish and grow in bone indicate that the attachment, maturation and activation of osteoclast precursors are likely to be important for tumour expansion, in which case the RANKL/RANK signalling system would be a suitable target for prevention and treatment. If the additional role of RANK-mediated cell motility proposed by Jones *et al.*[3] does indeed replace that of osteoclasts in the case of B16F10 melanoma cells, the provision of more convincing evidence would dispel any confusion.

T. John Martin*, Gregory R. Mundy†
*St Vincent's Institute of Medical Research, Fitzroy, Victoria 3065, Australia
e-mail: jmartin@svi.edu.au
†Vanderbilt Centre for Bone Biology, 1235 Medical Research Building IV, Nashville, Tennessee 37232-0575, USA

1. Teitelbaum, S. L. & Ross, F. P. *Nature Rev. Genet.* **4,** 638–649 (2003).
2. Mundy, G. R. *Nature Rev. Cancer* **2,** 584–593 (2002).
3. Jones, D. H. *et al. Nature* **440,** 692–696 (2006).
4. Thomas, R. J. *et al. Endocrinology* **140,** 4451–4458 (1999).
5. Arguello, F., Baggs, R. B. & Frantz, C. N. *Cancer Res.* **48,** 6876–6881 (1988).
6. Guise, T. A. *et al. J. Clin. Invest.* **98,** 1544–1549 (1996).
7. Gallwitz, W. E., Guise, T. A. & Mundy, G. R. *J. Clin. Invest.* **110,** 1559–1572 (2002).
8. Boyde, A., Maconacchie, E., Reid, S. A., Delling, G. & Mundy, G. R. *Scanning Electron. Micros.* **IV,** 1537–1554 (1986).

Competing financial interests: declared none.
doi:10.1038/nature05657

BONE METASTASIS

Jones et al. *reply*

Replying to: T. J. Martin & G. R. Mundy *Nature* 445, doi: 10.1038/nature05657 (2007)

Martin and Mundy[1] emphasize the crucial role played by osteoclasts in the metastasis of many tumour types to bone. Our intention was not to question the importance of osteoclasts in bone metastases, but rather to show that RANK ligand (RANKL) exerts additional action on cancer cells beyond its effects on osteoclasts[2]. We reported that epithelial tumour cells can express RANK, a finding that is supported by earlier work[3]; that RANKL has chemotactic activity[4]; and that *in vivo* inhibition of RANKL by osteoprotegerin reduces tumour burden in bone when assessed in a previously published experimental system[5].

Mundy and Martin[1] suggest that we cannot exclude the involvement of osteoclasts. For our *in vivo* studies, we chose a melanoma cell line that had previously been shown to metastasize to bone but not to trigger osteoclast activation[5]. We confirmed that spreading of these melanoma cells to bone does not result in obvious activation of osteoclasts by using several complementary approaches[2]: histomorphometry of bone structure; measurements of serum calcium, phosphorus, alkaline phosphatase and tartrate-resistant acid phosphatase; and radiographic analyses of bone. As these methods are the same as those used by Mundy and colleagues in their studies on bone metastases[6,7], our methodology would seem to be sufficient. Contrary to Mundy and Martin's assertion, details of the doses of zoledronic acid we used and the timing of treatment are provided in our Supplementary Information (page 6)[2].

Differences of opinion aside, Mundy and Martin pose an important question: what is the role of osteoclasts in bone metastases? All of the proposed theories are based on correlative localization studies and experiments with bisphosphonates that affect not only osteoclasts but also angiogenesis, apoptosis, proliferation and migration of other cell types[7–9]. This question can ultimately be answered only by the use of genetic models. Such experiments were not possible, however, as all mutant mice with disrupted osteoclasts have severely altered bone structures, or have other pathways that have been affected. To answer this question genetically, we have generated $RANK^{floxed}$ mice as a tool to knock out RANK in adult osteoclasts selectively, which should enable the role of osteoclasts in cancer metastases to be re-evaluated.

Our findings[2] describe an additional mechanism through which RANKL could promote bone metastases, namely the stimulation of cancer-cell migration. *RANKL* is the master gene for bone turnover through osteoclasts[10] and has a key function in epithelial-cell proliferation in the mammary gland[11]. Based

on our own and other evidence[2–4], including previous work by Martin and Mundy, RANKL qualifies as a bona fide soil factor that helps to explain the preferential metastasis of tumour cells into bones. The role of RANKL and RANK in cancer might extend beyond their function in osteoclasts and cell migration, to growth of the primary cancer. Because of its role in osteoclasts and in tumour cells, as also independently confirmed[1–4,10,11], inhibition of RANKL–RANK interaction offers a target for interfering with tumour metastasis and progression in bone.

D. Holstead Jones*†‡§, Tomoki Nakashima†, Otto H. Sanchez¶, Ivona Kozieradzki†‡§, Svetlana V. Komarova||, Ildiko Sarosi#, Sean Morony#, Evelyn Rubin‡§, Renu Sarao†, Carlo V. Hojilla¶, Vukoslav Komnenovic†, Young-Yun Kong, Martin Schreiber††, S. Jeffrey Dixon‡‡, Stephen M. Sims‡‡, Rama Khokha‡¶, Teiji Wada†, Josef M. Penninger†‡§**

*Faculty of Health Sciences, University of Ontario Institute of Technology, Oshawa, Ontario L1H 7K4, Canada
†Institute of Molecular Biotechnology of the Austrian Academy of Sciences, 1030 Vienna, Austria
e-mail: josef.penninger@imb.oeaw.ac.at
‡Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 2C1, Canada
§Department of Immunology, University of Toronto, Toronto, Ontario M5G 2C1, Canada
¶Ontario Cancer Institute, University Health Network, Toronto, Ontario M5G 2C1, Canada
||Faculty of Dentistry, McGill University, Montreal, Quebec H3A 1A4, Canada
#Amgen Inc., Thousand Oaks, California 91320-1799, USA
**Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang, 790-784 Kyungbuk, South Korea
††Medical University of Vienna, Department of Obstetrics and Gynecology, Waehringer Guertel 18-20, 1090 Vienna, Austria
‡‡CIHR Group in Skeletal Development and Remodeling, Department of Physiology and Pharmacology and Division of Oral Biology, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Ontario N6A 5C1, Canada

1. Mundy, T. J. & Mundy, G. R. *Nature* **445,** doi: 10.1038/nature05657 (2007).
2. Jones, D. H. *et al. Nature* **440,** 692–696 (2006).
3. Thomas, R. J. *et al. Endocrinology* **140,** 4451– 4458 (1999).
4. Mosheimer, B. A., Kaneider, N. C., Feistritzer, C., Sturn, D. H. & Wiedermann, C. J. *Arthr. Rheum.* **50,** 2309–2316 (2004).
5. Sanchez-Sweatman, O. H., Lee, J., Orr, F. W. & Singh, G. *Eur. J. Cancer* **33,** 918–925 (1997).
6. Sterling, J. A. *et al. Cancer Res.* **66,** 7548–7553 (2006).
7. Hiraga, H., Williams, P. J., Mundy, G. R. & Yoneda, T. *Cancer Res.* **61,** 4418–4424 (2001).
8. Clezardin, P. *Cancer Treat. Rev.* **31** (suppl), 1–8 (2005).
9. Wood, J. *et al. J. Pharmacol. Exp. Ther.* **302,** 1055–1061 (2002).
10. Kong, Y.-Y. *et al. Nature* **397,** 315–323 (1999).
11. Fata, J. E. *et al. Cell* **103,** 41–50 (2000).

doi:10.1038/nature05658