Most patients with cancer die not because of the tumour in the primary site, but rather because it has spread to other sites. It is difficult to determine, however, precisely how frequently different tumours metastasize to bone. Patients with advanced breast and prostate cancers almost always develop bone metastases, and the chances are high that, in patients who are originally diagnosed with breast or prostate cancers, the bulk of the tumour burden at the time of death will be in bone. How long the patient lives with the tumour is likely to influence whether bone metastases will occur. For example, in patients who quickly succumb to cancer, due to an aggressively growing primary tumour, bone metastases will be relatively uncommon simply because they have not had time to develop. This does not mean that the tumour cells did not have the potential to grow in bone.

There are no reliable prevalence figures for people with bone metastases, but estimates can be made. Of the four million people who die in the United States each year, approximately one-quarter die from cancer, and 70% of these have either breast, lung or prostate cancer. So, there are probably more than 350,000 people in the United States who die each year with bone metastases, and probably two to three times this number if patients in the European Union and Japan are also included. The number of bone metastases increases when we consider patients who are living with the condition, as breast and prostate patients cancer frequently live longer than one year.

Bone metastases are infrequently silent — they are usually associated with severe bone pain, which can be intractable. The mechanisms responsible for bone pain are poorly understood, but seem to be a consequence of osteolysis (bone breakdown). There is evidence that bone resorption inhibitors, such as osteoprotegrin (OPG) or bisphosphonates, might be used to alleviate bone pain (BOX 1). Osteolysis is also accompanied by increased bone fragility; susceptibility to fracture is markedly increased, and pathological fractures frequently occur as a consequence of bone metastases. They often occur in load-bearing bones, and are a particular treatment problem when they are present in the neck or shaft of the femur, or in the pelvis. Other consequences of bone metastasis are leukoerythroblastic anaemia, bone deformity, hypercalcaemia, and nerve-compression syndromes such as spinal-cord compression (BOX 2).

It has been traditional to think of bone metastases as either osteolytic or osteoblastic (FIG. 1), with entirely different factors being responsible for each. From this viewpoint, osteolytic metastases are believed to be caused by osteoclast-activating factors — the most important of which might be parathyroid-hormone-related peptide (PTHrP) — and which are released by tumour cells in the bone microenvironment. Osteoblastic metastases, conversely, are believed to be caused by cancer-cell production of factors that stimulate osteoblast proliferation, differentiation and bone formation.
Summary

- Common tumours, such as those of the breast, lung and prostate, frequently metastasize to bone, and in many patients with advanced disease the skeleton is the site of the most significant tumour burden.
- There are different patterns of bone effects in patients with cancer, ranging from purely or mostly destructive or osteolytic (breast cancer, myeloma) to mostly forming or osteoblastic (prostate cancer).
- In the case of breast-cancer-causing osteolysis, the main mediator is parathyroid-hormone-related peptide (PTHrP), whereas, in osteoblastic lesions, known mediators include endothelin-1 and platelet-derived growth factor.
- In osteolytic metastasis, there is a vicious cycle in the bone microenvironment, whereby bidirectional interactions between tumour cells and osteoclasts lead to both osteolysis and tumour growth.
- The molecular mechanisms that are responsible for this vicious cycle are now being clarified and involve tumour-cell production of PTHrP and bone-derived growth factors that are released as a consequence of increased bone resorption.
- Bisphosphonates interrupt the vicious cycle and cause not only reduction in osteolytic bone lesions, but also decrease the tumour burden in bone.
- More effective treatments for interruption of the vicious cycle are now being developed, including specifically neutralizing antibodies to PTHrP and more efficacious osteoclast inhibitors.

Osteolytic and osteoblastic lesions

Although breast and prostate cancer are the two tumour types that most commonly metastasize to bone, the end result of metastasis by each is usually quite different. In breast cancer, bone metastases are predominantly osteolytic. Osteolysis is caused by osteoclast stimulation — not by the direct effects of cancer cells on bone. Although the dominant lesion is lytic and destructive, there is usually also a local bone-formation response, which presumably represents an attempt at bone repair. This increase in bone formation in patients with osteolytic lesions is reflected by increased levels of serum alkaline phosphatase — a marker of osteoblast (bone-forming cell) activity — and increased uptake of bone-scanning agents at the site of the lesion. However, despite this secondary increase in local bone formation, the predominant effect is osteolysis.

In prostate cancer, alternatively, bone metastases are frequently osteoblastic. In these metastases, there is a profound local stimulation of osteoblasts adjacent to the metastatic tumour cells, as measured by alkaline phosphatase and osteocalcin levels. Up to 25% of patients with bone metastases from breast tumours, however, also have blastic lesions that are similar to those with metastatic prostate cancer, and some patients with prostate cancer have osteolytic lesions that are similar in nature to those seen in patients with metastatic breast cancer.

So the concept that there are basically two types of bone metastases is probably too simplistic. The processes of bone resorption and bone formation are almost always linked or coupled — although this coupling might be distorted in cancer. Recent observations indicate that there is a spectrum of bone metastasis. At one end, predominantly osteolytic lesions are associated with reduced osteoblast activity that is uncoupled from rates of bone resorption. Bone metastases that are predominantly osteoblastic, alternatively, also have resorptive components. There is much evidence that both resorption and formation are activated in most bone metastases. In addition to the increased uptake of bone-scanning agents at the metastatic site (a reflection of osteoblastic activity), serum markers of bone resorption, such as urinary hydroxyproline, deoxypyridynoline and pyridinoline crosslinks, are also frequently increased.

Researchers have followed the course of development of osteoblastic metastases from human breast cancer xenografts in nude mice. This model allows sequential morphological observations of the metastases — information that is impossible to obtain from clinical studies. In this model, metastatic tumours are initially osteolytic — characterized by increases in osteoclast activity, which is associated with increased production of bone-resorption markers. This lytic activity is then followed by a wave of bone formation, and osteoclast activity is reduced. The precise molecular mechanisms that are responsible for this switch are not known. These studies, however, have important implications for therapeutic strategies. Treatments that are aimed at inhibiting bone resorption, such as bisphosphonates, might be effective not only in treating lesions that are primarily osteolytic, but also in treating osteoblastic metastases — if the osteoblastic response is dependent on previous osteoclastic activity.

Some types of bone metastases are known to always be osteolytic. Myeloma cells cause exclusively osteolytic bone lesions, and although myeloma is not strictly a metastasis, there are probably solid tumours that behave in a similar manner. There are also some osteoblastic lesions that seem to have little or no resorptive component. Charhon et al. described some human cancers that cause osteoblastic metastases with...
tumour cells and the normal host cells, and each is a potential target for the development of drugs that are designed to abrogate the metastatic process.

no morphological evidence of a resorptive component. So, how do cancer cells metastasize to bone and, when they have reached their destination, how do they set up this cycle of bone formation and destruction?

Pathophysiology of bone metastasis
The initial steps in the development of bone metastases are similar to those of metastases to any other site. Primary tumour cells invade their surrounding normal tissue by producing proteolytic enzymes, which traverse the walls of small blood vessels in the normal tissue or those induced by the tumour and enter the circulation. They then travel to distant organ sites. These events have been described as inefficient, in that many cancer cells do not survive the normal protective host-surveillance mechanisms during these initial stages.

The cancer cells that do survive can enter the wide channelled sinuses of the bone-marrow cavity and are positioned to become bone metastases. Cancer cells must possess certain properties for this to occur. They must have the capacity to migrate across the sinusoidal wall, invade the marrow stroma, generate their own blood supply and travel to the endosteal bone surface. At this site, they stimulate the activity of osteoclasts or osteoblasts, thereby determining whether the subsequent bone metastasis is osteolytic or osteoblastic. Each of these steps involves important molecular interactions between the tumour cells and the normal host cells, and each is a potential target for the development of drugs that are designed to abrogate the metastatic process.

Figure 1 | Types of bone metastasis. Bone metastasis is often classified as either a osteolytic or b osteoblastic, and one of these effects is usually predominant. For example, metastases from breast and lung tumours are generally osteolytic, whereas metastases from prostate cancer are generally osteoblastic. However, most blastic metastases have a resorptive component, and most lytic lesions are accompanied by some attempt, albeit incomplete, of repair or bone formation.

Hypercalcaemia
Hypercalcaemia (increased blood-calcium concentration) is an important complication of osteolytic bone disease. It occurs relatively frequently in patients with extensive bone destruction, and is particularly common in breast, lung, renal, ovarian and pancreatic carcinomas, as well as in myeloma. It is distressing for the patient, and it must be recognized and treated vigorously.

Hypercalcaemia that occurs in most patients with cancer is due to the production of the peptide parathyroid hormone-related peptide (PTHrP) by the tumour. PTHrP acts on PTH receptors to cause increased bone resorption and increased renal tubular calcium reabsorption. Bone destruction is an important cause of hypercalcaemia, but the important contributing role of renal mechanisms has been under-appreciated. Hypercalcaemia occurs as a consequence of the combination of these effects and by overwhelming of the calcium homeostatic defence mechanisms. This can be appreciated when the calcium homeostasis for a normal adult in zero calcium balance is considered (see figure). The numbers are estimates of the amount of calcium that is exchanged between the extracellular fluid and gut, kidney and bone each day.

Bisphosphonates — a class of drugs that block bone resorption — have made an enormous difference to both the frequency and management of hypercalcaemia in patients with cancer. First, they have reduced its occurrence. And second, when hypercalcaemia does occur, it is readily treated, at least initially, and nearly all patients show a beneficial response. However, we may have become complacent in the treatment of hypercalcaemia of malignancy. Bisphosphonates might have only a transient beneficial effect because renal tubular calcium reabsorption is unaffected by bisphosphonates. This is not apparent in most patients because hypercalcaemia is usually a hallmark of extensive tumour burden and advanced disease, and many patients with hypercalcaemia die within one month of its onset.

Neutralizing antibodies to PTHrP have been shown in preclinical studies to be very effective in the treatment of hypercalcaemia.
Mechanisms of osteolytic metastasis

The mechanisms by which cancer cells cause osteolytic metastasis are gradually being unravelled. In metastatic human breast cancer, the peptide PTHrP is the main mediator of osteoclast activation, and human osteolytic breast cancer cells have been shown to express PTHrP in vivo. PTHrP expression is greater when the tumour cells are present at the metastatic bone site than when they are present in soft-tissue sites or in the breast. Immunohistochemistry and in situ hybridization experiments have shown that breast cancer metastases in bone express higher levels of PTHrP than cells that have metastasized to soft tissue or that are present in the primary site. This indicates that PTHrP is a specific mediator of osteolysis in metastatic breast cancer, and it is likely to be the mediator of bone destruction in most other osteolytic cancers.

The role of PTHrP in inducing osteolysis is, however, complex. Henderson et al. have recently published a clinical study showing that PTHrP expression by primary tumours is associated with a favourable outcome and less propensity to bone metastasis. Other preclinical and clinical data, however, associate PTHrP production with bone metastatic potential. This could be due to the fact that tumour cells that express high levels of PTHrP are selected for their ability to metastasize to bone, or that the bone microenvironment increases expression of PTHrP from cancer cells that have spread there. Data from Henderson et al. support the latter explanation. PTHrP that is expressed by prostate cancer cells has also been reported to have anabolic effects on bone. It is possible that this tumour peptide could promote osteoblastic metastases that are associated with prostate cancers, although there is no direct data to support this.

Increased expression of PTHrP is not the only phenotypic change that occurs in breast cancer cells that enter the bone microenvironment. Mutations in genes that encode mutant oestrogen receptors, interleukin (IL)-8 and the receptor for PTH have also been associated with bone metastasis. Tumour cells that reside in different metastatic sites might have subtle differences in phenotype that could affect not just the behaviour of the cells at that site, but also responses to therapy.

So, is PTHrP a viable therapeutic target for bone metastases? Osteolysis caused by human breast cancer metastases was shown to be blocked by neutralizing antibodies against PTHrP. Furthermore, compounds that specifically decrease PTHrP expression have been shown to inhibit osteolysis caused by human breast cancer cells in vivo.

Blocking PTHrP might also have other clinical benefits. Ogata et al. have proposed that this peptide might also be associated with cachexia.

RANKL and osteolytic bone disease. PTHrP stimulates osteoclast activity by stimulating production of the cytokine RANKL (receptor activator of nuclear factor-κB ligand), which binds and activates its receptor, RANK, which is expressed by osteoclasts. There is, however, a debate over the exact function of RANKL in the osteolytic bone activity that is associated with human solid cancers or myeloma. RANKL production by stromal cells is a final common mediator of osteoclast activity that is stimulated by several factors. Many researchers have reported that RANKL is expressed by tumour cells in the bone microenvironment, but it is not clear whether the production of RANKL by tumour cells
Mechanisms of osteoblastic metastasis

There is accumulating data to identify the factors that stimulate bone formation that is associated with metastatic tumours (Box 3). One of the most well-studied mediators is the ubiquitous growth factor endothelin-1, which stimulates bone formation and osteoblastic proliferation in bone organ cultures. Endothelin-1 is increased in the circulation of patients with osteoblastic metastases and prostate cancer and is also expressed by breast cancer cell lines that cause osteoblastic metastases. Osteoblast proliferation and bone metastases have both been shown to be inhibited in vivo by endothelin-A-receptor antagonists. Several other factors, as described below, have also been proposed to be potential mediators of osteoblastic metastasis that is associated with prostate cancer (Fig. 4).

The transforming growth factor-β family

Several members of the transforming growth factor-β (TGF-β) family are powerful in vivo stimulators of new bone formation, and are candidate mediators of osteoblastic metastasis. TGF-β2 is expressed at high levels by PC3 human prostate cancer cells, and was originally isolated from the human prostate cancer cell line PC3 (REF. 36). TGF-β2 stimulates the proliferation of osteoblasts in vitro, as well as bone formation in vivo. Both normal, and neoplastic human and rat prostate tissues also express a variety of bone morphogenetic proteins (BMPs) — namely, BMP2, BMP3, BMP4 and BMP6 mRNA. Several other factors, such as BMP2, and BMP6, have been shown to have mitogenic activity for osteoblasts. The carboxy-terminal proteolytic domain might mediate tumour invasiveness or growth-factor activation. Proteases that activate growth factors such as TGF-β have been shown to have important functions in bone

Proteases and their activators

There have been several reports that a mitogen for rat calvarial osteoblastic cells has been purified from the conditioned media of PC3 cells, and that the sequences of the first ten amino acids were identical to that of the serine protease uPA. Overexpression of uPA by rat prostate cancer cells has been shown to induce bone metastases in vivo, and an amino-terminal fragment of uPA has been shown to have mitogenic activity for osteoblasts. The carboxy-terminal proteolytic domain might mediate tumour invasiveness or growth-factor activation. Proteases that activate growth factors such as TGF-β have been shown to have important functions in bone.
The cellular events that are responsible for bone formation include osteoblast proliferation and differentiation, as well as osteoclast apoptosis. Osteoblast proliferation is driven by mitogenic factors, such as transforming growth factor-β (TGF-β), insulin-like growth factors (IGFs) and fibroblast growth factors (FGFs). Osteoclast apoptosis is induced by TGF-β, but also by drugs such as oestrogen and bisphosphonates. During physiological bone remodelling, the release of TGF-β as a consequence of resorption might be the physiological inducer of osteoclast apoptosis and, therefore, be responsible for limiting osteoclast lifespan and impairing continued resorption. Osteoblast differentiation involves expression of the structural proteins of the bone matrix, such as type I collagen, as well as other bone proteins, including alkaline phosphatase and osteocalcin. The bone morphogenetic proteins (BMPs) act predominantly to stimulate osteoblast differentiation. Bone formation involves a cascade of events that, once triggered, continues until the osteoblasts undergo apoptosis, which might be regulated by ambient growth-factor concentrations and influenced by drugs such as corticosteroids and parathyroid hormone. In cancer patients who have osteoblastic metastases, any of these growth factors that are released by the tumour cells could be expected to ultimately cause osteoblast differentiation and subsequent bone formation.

**Bone growth factors and bone remodelling**

The concept that there is a relationship between the seed (tumour cells) and the soil (metastatic site) that determines a cancer’s capacity to grow and thrive was first proposed by Stephen Paget more than 100 years ago. In the case of bone metastasis by breast cancer cells, we now understand some of the molecular mechanisms that support this concept. As discussed above, breast cancer cells, when present in the bone microenvironment, overproduce PTHrP, and this leads to osteoclastic bone resorption. Consequently, active growth factors are released from bone that cause proliferation of breast cancer cells. This stimulates further production of PTHrP, which, in turn, causes more bone loss. Studies have also shown that IGF1, which is released during bone resorption, can also induce tumour-cell proliferation under similar circumstances. In this way, a vicious cycle (FIG. 5) is set up between the tumour cells and bone resorbed bone releases TGF-β and IGF1, thereby stimulating tumour-cell proliferation and further PTHrP release, which, in turn, causes more bone

**Growth factors.** Prostatic cancer cells express large amounts of both acidic and basic fibroblast growth factors (FGFs) and, which are potential mediators of osteoblast proliferation in patients with prostate cancer. Both acidic (FGF1) and basic FGF (FGF2) stimulate bone formation in vivo. Ibicka et al. have shown that a human tumour cell line that produces an extended form of FGF2 activates osteoblasts and causes bone formation in vivo.

When the human breast cancer cell line MCF-7 is transfected stably with the ERBB2 (also known as HER2/Neu) proto-oncogene, it causes osteoblastic metastases in mice. These tumour cells have been shown to produce the B isoform of platelet-derived growth factor (PDGF-BB). Conditioned media from these tumour cells promotes bone formation in bone organ cultures, but media from cells that are stably transfected with antisense oligonucleotides to PDGF-BB do not. This work indicates that PDGF-BB is a potential mediator of the osteoblastic response in some tumour types.

**The bone microenvironment**

Why do some cancers have such high avidity for bone — or any other specific metastatic site, for that matter? One reason might be that most circulating tumour cells pass through the bone marrow, as a consequence of its vascularity. However, there are other highly vascularized organs to which tumour cells rarely metastasize. It is, therefore, probable that the environment of bone provides a particularly fertile ground for the growth and aggressive behaviour of the tumour cells that reach it.

The concept that there is a relationship between the seed (tumour cells) and the soil (metastatic site) that determines a cancer’s capacity to grow and thrive was first proposed by Stephen Paget more than 100 years ago. In the case of bone metastasis by breast cancer cells, we now understand some of the molecular mechanisms that support this concept. As discussed above, breast cancer cells, when present in the bone microenvironment, overproduce PTHrP, and this leads to osteoclastic bone resorption. Consequently, active growth factors are released from bone that cause proliferation of breast cancer cells. This stimulates further production of PTHrP, which, in turn, causes more bone loss. Studies have also shown that IGF1, which is released during bone resorption, can also induce tumour-cell proliferation under similar circumstances. In this way, a vicious cycle (FIG. 5) is set up between the tumour cells and bone resorbed bone releases TGF-β and IGF1, thereby stimulating tumour-cell proliferation and further PTHrP release, which, in turn, causes more bone

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**Figure 4 | Model for osteoblastic bone metastases caused by prostate cancer.** The production of factors, such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), by tumour cells can directly stimulate osteoblast activity and subsequent bone formation. Proteases, such as prostate-specific antigen, and protease activators, such as urokinase (uPA), can activate latent TGF-β, release IGFs from inhibitory binding proteins (IGFBPs) and inactivate the osteolytic factor parathyroid-hormone-related peptide (PTHrP) to promote bone formation.
resorption and subsequent release from the resorbed bone matrix of TGF-β and IGF1.

The evidence for this concept comes from a series of studies. First, when human breast cancer cells are inoculated into the left ventricle of nude mice, they cause osteolysis in distant skeletal sites that is abrogated by neutralizing antibodies to PTHrP. PTHrP is also produced in greater amounts by breast cancer cells that have metastasized to bone than those that have metastasized to other sites. Alternatively, TGF-β increases the production of PTHrP by breast cancer cells. Breast cancer cells that are stably transfected with mutant TGF-β receptors that cannot respond to TGF-β do not produce PTHrP, and have markedly reduced osteolytic lesions following inoculation in nude mice. IGF1 also promotes breast cancer cell proliferation, whereas mutant IGF1 receptors reduce the proliferation of breast cancer cells in bone.

Therapeutic approaches

The concept of the vicious cycle alters the approach to the treatment of bone metastases, because it means that osteolysis inhibitors might also decrease bone tumour burden. This concept has recently been supported by in vivo studies. Bisphosphonates, when administered to mice with osteolytic lesions following inoculation of MDAMB-231 cells, have decreased bone lesions, as expected, but also a decrease in tumour burden. Similarly, mice that are treated with neutralizing antibodies to PTHrP, as well as tumours that express mutant TGF-β receptors experience a decrease in tumour burden. There is a rationale for developing clinical inhibitors of the bone resorption process, as so much data now indicates that osteolysis supports the growth and aggressive behaviour of metastatic cancers.

Diel et al. and Powles et al. have provided clinical data to support the idea that osteolysis inhibitors also reduce tumour burden in bone. However, there is residual controversy over whether the same effects can be seen in soft-tissue metastases. Studies by Diel and colleagues indicate that osteolysis inhibitors do slow tumour growth in these sites, whereas a study by Powles et al. refutes this idea. One study has reported that bisphosphonates can actually promote soft-tissue metastasis. This important issue will probably require much larger studies to be resolved definitely.

Preclinical data indicate that the bisphosphonates have no effect on soft-tissue metastases if they are administered after metastases are already established. However, if they are given from the time of tumour-cell inoculation, they might increase soft-tissue metastases, presumably by rendering bone unsuitable as a site for metastatic growth. Although definitive recommendations cannot be made until clinical data are available, this data indicate that, although bisphosphonates can be safely administered once metastases are already present, they should be used prophylactically with great caution.

Newer bone-resorption inhibitors (Table 1) might be even more effective than bisphosphonates. These

<table>
<thead>
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<th>Therapy</th>
<th>Mechanism</th>
<th>Stage of clinical development</th>
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<tbody>
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<td>Bisphosphonates</td>
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<td>On the market</td>
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<tr>
<td>Osteoprotegerin</td>
<td>Prevents RANKL from binding its receptor and stimulating osteoclasts</td>
<td>Phase II</td>
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<tr>
<td>RANK-Fc</td>
<td>Prevents RANKL from binding its receptor and stimulating osteoclasts</td>
<td>Phase I</td>
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<tr>
<td>PTHrP antibodies</td>
<td>Neutralize PTHrP</td>
<td>Phase III</td>
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<tr>
<td>Vitamin-D analogues</td>
<td>Decrease PTHrP production</td>
<td>Phase III</td>
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Table 1 | Approaches to treating bone metastases

Figure 5 | The ‘vicious cycle’ hypothesis of osteolytic metastases. Interactions between tumour cells and osteoclasts cause not only osteoclast activation and subsequent bone destruction, but also aggressive growth and behaviour of the tumour cells. Production of the parathyroid-hormone-related protein (PTHrP) by tumour cells enhances osteoclast activation and osteolysis (see Fig. 4). Osteolysis leads to the release of bone-derived growth factors, including transforming growth factor-β (TGF-β) and insulin-like growth factor 1 (IGF1), and raises extracellular calcium (Ca2+) concentrations. The growth factors bind to receptors on the tumour-cell surface and activate autophosphorylation (P) and signalling through pathways that involve SMAD (cytoplasmic mediators of most TGF-β signals) and mitogen-activated protein kinase (MAPK). Extracellular Ca2+ binds and activates a Ca2+ pump. Signalling through these pathways promotes tumour-cell proliferation and production of PTHrP. Other cytokines might also be involved, such as interleukin (IL)-6, IL-1, IL-11 and IL-18 (not shown). There are many potential sites for the production of tumour-cell cytokines in bone, including bone matrix of TGF-β and IGF1. There is a rationale for developing clinical inhibitors of the bone resorption process, as so much data now indicates that osteolysis supports the growth and aggressive behaviour of metastatic cancers.
include direct inactivators of osteoclast activity. For example, OPG — the natural decoy receptor to RANK — is an extremely powerful and potent inhibitor of bone resorption. RANK-Fc is a hybrid chimeric molecule that acts in an identical way to OPG. These agents cause the most profound decreases in osteoclastic bone resorption, and can be expected to cause similar effects on tumour burden as do the bisphosphonates. In the case of osteoblastic metastasis, the early preclinical studies of Guise and colleagues indicate that specific inhibitors of endothelin-1 signalling, namely antagonists of the endothelin-A receptor, have beneficial effects on both the bone lesions and the tumour burden.

**Future research**

**Osteolysis in other cancers.** So far, there has been little detailed examination of the factors that might be responsible for osteolysis in tumours other than breast cancer or myeloma. A series of cytokines, including RANK ligand and macrophage inflammatory protein 1α (MIP1α), have been proposed to promote osteolysis by melanoma cells. However, these are probably not the only cytokines that promote bone destruction that is associated with myeloma. It has been indicated that IL-1, lymphotoxin, IL-6, hepatocyte growth factor and PTHrP might all have a subsidiary role. In other cancers, factors such as IL-11, IL-18, TGF-β, and even prostaglandins might be produced by tumours or by host cells that are activated at the tumour site and are involved in the pathophysiology of hypercalcaemia. There is data that inhibitors of prostaglandin synthesis might be effective at blocking tumour-induced osteolysis in some cancers.

Will bisphosphonates be useful in treating bone metastases that are associated with tumours other than breast cancer, and will any form of bisphosphonate be satisfactory as a treatment for metastasis? These questions cannot be answered definitively at the present time, but it does seem likely that bisphosphonates will also be effective treatments for bone metastases from other cancer types. This is because the cellular mechanisms that are responsible for osteolysis are fundamentally identical, and should be blocked in a similar manner by similar agents.

There have been suggestions that some bisphosphonates induce tumour-cell apoptosis, and that this might be a mechanism for decreasing tumour burden in the metastatic site. Evidence that bisphosphonates cause apoptosis comes from in vitro studies that involve relatively high concentrations of the drugs, but further studies are required to determine the direct effects of bisphosphonates on cancer cells.

**Osteolytic and osteoblastic factors.** Many of the factors that promote osteoblastic metastasis remain to be identified. At present, most evidence supports a role for endothelin-1 in breast cancer metastasis, but there is less convincing data to support its involvement in prostate cancer metastasis — due, in part, to the relative absence of animal models of this disease. Many other factors — including PDGF, members of the TGF-β family, growth factors and proteolytic systems — are also involved, possibly all in the same tumour, but more work needs to be done to tie these mechanisms together. So far, TGF-β and IGF1 have been identified as the bone-derived factors that are involved in the vicious cycle of bone destruction and tumour growth. Other growth factors that are present in the bone microenvironment and released as a consequence of bone resorption, such as the PDGFs, BMPs, FGFs and possibly even extracellular calcium might also be involved. It is, in fact, probable that the end result is a combined effect of several factors that act synergistically.

Tumour cells in the bone microenvironment have an altered phenotype, compared to the same cells in the primary site or other soft-tissue sites. The best evidence for this is in the increased expression of PTHrP in bone metastases. There are, however, other genes, including the PTH receptor and the estrogen receptor, that have altered gene expression. TGF-β receptors might be particularly important, because the presence or abundance of TGF-β receptors might be the main influence on the aggressive behaviour of the tumour cell. For example, one explanation for the dormancy that occurs in some tumours might be low expression levels of TGF-β receptors on tumour-cell surfaces during the dormant phase.

**Mechanisms of abnormal coupling.** Little is understood of the molecular mechanisms that are responsible for the balanced coupling between bone resorption and bone formation that occurs under normal physiological conditions. Furthermore, nothing is known of the mechanisms that are responsible for the distortion in this process that occurs in metastatic cancer growing in bone. Perhaps clarification of the aberrations in the coupling that is responsible for predominantly osteolytic or osteoblastic lesions will also shed light on the mechanisms that are responsible for normal bone remodelling.

Metastasis is the single most catastrophic complication of cancer, and understanding the biology of the process should provide not only greater insights into normal cell behaviour, but also lead to new therapies that are specifically designed to limit or prevent this cause of morbidity and mortality in patients with cancer. Our understanding of the cellular and molecular events is improving significantly, and the possibility of such therapy is becoming more realistic.
39. Ogata, E. PTHrP, paraneoplastic syndrome and cancer


The classical paper describing the ‘seed and soil’ hypothesis of tumour-cell metastasis.


50. In a preclinical model of human breast cancer metastasis to bone, bisphosphonates reduce not only osteolysis but also tumour burden.


The authors show, in a controversial study, that the bisphosphonate clodronate reduces tumour burden in both bone and non-osseous metastatic sites in patients with breast cancer.


**Online links**

**DATABASES**

The following terms in this article are linked online to:


- alendronate | pamidronate


**FURTHER INFORMATION**

Access to this interactive links box is free online.

- University of Michigan Comprehensive Cancer Center — Bone Metastasis Facts: [http://www.cancer.med.umich.edu/learn/bonemetastasis.htm](http://www.cancer.med.umich.edu/learn/bonemetastasis.htm)
Biog
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Endocrinology and Metabolism at the University of Texas Health
Science Center (1980–2001). His research interests include drug dis-
covery in osteoporosis and the effects of tumours on the skeleton.

Databases
Cancer.gov
bone cancer
http://www.cancer.gov/cancer_information/doc_pdq.aspx?ver-
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breast cancer
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lung cancer

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LocusLink
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BMP2

BMP3

BMP4

BMP6

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endothelin-1

endothelin-A receptor

ERBB2

FGF1

FGF2

hepatocyte growth factor

IGF1

IGFBPs
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=igfbp%20not%201490%20not%203491%20not%202274%20not%205155&ORG=Hs

IKK
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=ikk%20not%20similar%20not%206orf5%20not%2011140%20not%205155&ORG=Hs

IL-1
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=il1%20not%20myd88%20not%20traf66&ORG=Hs

IL-6

IL-8
IL-11

IL-18

JUN

lymphotoxin
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=lymphotoxin%20traf5%20tbr6&ORG=Hs

MAPK
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=mapk1%20mapk2%20mapk3%20mapk4%20mapk5%20mapk6%20mapk7%20mapk8%20mapk9%20mapk10%20mapk11%20mapk12%20mapk13%20mapk14&ORG=Hs

MIP1α

NF-κB
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=NFkB%20CARD10%20OC51580%20TANK%20TNFRSF11A&ORG=Hs

oestrogen receptor

OPG

ostecocalcin

PDGF
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=pdgfa%20pdgfb%20pdgfc%20201277%202011334&ORG=Hs

PSA

PTHrP
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=pthr1%20pthr2&ORG=Hs

RANK

RANKL

SMAD
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=smad1%20smad2%20smad3%20smad4%20smad5%20smad6%20smad7%20smad8%20smad9%20smad10%201634%2020033%20204609%20206498&ORG=Hs

TGF-α

TGF-β
http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi?Q=tgfb1%20tgfb3%20eng%2020tgfb1%20tgfb3%20tgfb2&ORG=Hs

TGF-β2

uPA

Medscape Drug Info
alendronate
http://promini.medscape.com/drugdb/drug_pharmacology_chemistry.asp?DrugCode=1%2D10124&DrugName=ALEN-DRONATE+SODIUM+ORAL&DrugType=1

pamidronate
http://promini.medscape.com/drugdb/drug_pharmacology_chemistry.asp?DrugCode=A%2D6250&DrugName=PAMIDRONE+DI+SODIUM+INTRAVEN%2E&DDrugType=1

OMIM
Paget's disease
Osteoporosis and other diseases of bone loss are a major public health problem. Here it is shown that the statins, drugs widely used for lowering cholesterol, also enhance new bone formation in vivo and in rodents. This effect was associated with increased expression of the bone morphogenetic protein–2 (BMP–2) gene in bone cells. Lovastatin and simvastatin increased bone formation when injected subcutaneously over the calvaria of mice and increased cancellous bone volume when orally administered to rats. Thus, in appropriate doses, statins may have therapeutic applications for the treatment of osteoporosis.
have cellular effects independent of the cholesterol biosynthesis pathway (8). Cultured murine (2T3) or human (MG-63) bone cells exposed to statins showed enhanced expression of BMP-2 mRNA, as assessed by Northern (RNA) blot analysis (Fig. 1). This effect appeared to be specific, because the statins did not alter expression from the BMP-4 promoter (Fig. 1) or from promoters derived from the gene encoding interleukin-6, the gene encoding parathyroid hormone (PTH)–related peptide, or from SV40 and cytomegalovirus. A sandwich enzyme-linked immunosorbent assay for BMP-2 revealed increased protein production in MG-63 cells incubated with simvastatin (a 2.7-fold increase with 2.5 μM simvastatin).

To investigate the biological effects of statins on bone, we added them to neonatal murine calvarial (skullcap) bones in organ culture (9, 10). Calvaria from 4-day-old pups of Swiss white mice (Harlan Sprague-Dawley) were explanted, dissected free of adjacent connective tissue, placed in tissue culture medium containing 0.1% bovine serum albumin (BSA), and incubated with test compounds for 3 to 7 days. The amount of new bone formation was then assessed morphologically as described in (10) and Table 1. Lovastatin, simvastatin, fluvastatin, and mevastatin each increased new bone formation by approximately two- to threefold, an increase comparable to that seen in this assay after treatment with BMP-2 and fibroblast growth factor–1 (FGF-1). There was also a striking increase in new bone and in osteoblast cell numbers at all stages of differentiation (Fig. 2, A and B, and Table 1).

We next injected lovastatin and simvastatin into the subcutaneous tissue overlying the murine calvaria in vivo (11–14). The bone cells of the calvaria are responsive to both bone-resorbing factors and osteoblast-stimulating factors (10–15). This technique requires reproducible placement of small volumes of factors or compounds adjacent to bone. It is minimally invasive, and the calvarial periosteum is not scraped or damaged. Male Swiss ICR (Institute for Cancer Research) white mice, 4 to 5 weeks of age, were injected three times per day for 5 days over the right side of the calvaria with either vehicle or the test compound. Each injection contained the compound dissolved in 50 μl of phosphate-buffered saline (PBS) with 2% dimethylsulfoxide and 0.1% BSA. Mice were killed on day 21, and calvaria were removed for histomorphometric analysis. We observed an almost 50% increase in new bone formation after only 5 days of treatment, again comparable to that seen with FGF-1 (Fig. 3, A and B) and BMP-2. However, FGF-1 also increases proliferation of cells in the overlying subcutaneous tissue, an effect not seen with BMP-2 or the statins (Fig. 3).

To determine whether the statins stimulate new bone formation when administered systematically, we tested their effect on trabecular bone. It is minimally invasive, and the calvarial periosteum is not scraped or damaged. Male Swiss ICR (Institute for Cancer Research) white mice, 4 to 5 weeks of age, were injected three times per day for 5 days over the right side of the calvaria with either vehicle or the test compound. Each injection contained the compound dissolved in 50 μl of phosphate-buffered saline (PBS) with 2% dimethylsulfoxide and 0.1% BSA. Mice were killed on day 21, and calvaria were removed for histomorphometric analysis. We observed an almost 50% increase in new bone formation after only 5 days of treatment, again comparable to that seen with FGF-1 (Fig. 3, A and B) and BMP-2. However, FGF-1 also increases proliferation of cells in the overlying subcutaneous tissue, an effect not seen with BMP-2 or the statins (Fig. 3).

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Control lumbar vertebrae were embedded in paraffin. The proximal end of the tibia and the vertebrae were removed and fixed in formalin. Right tibia, right femur, and lumbar were killed by anesthetic overdose, and the completion of the experiment, the rats were administered statins to ovariectomized rats as frequently in postmenopausal women, we administered statins to ovariectomized rats as well as rats with intact ovaries (Table 2). At recent studies, it has been shown that certain bone volume in female rats after oral administration of statins (experiment 1) and lovastatin (experiment 2) on neonatal murine calvarial bone organ cultures. Explanted bones were cultured for 72 hours, and four bones were in each treatment group. Values are means ± SEM.

| Treatment         | Simvastatin | Lovastatin | Statin | myocardial infarction (MIs) over the murine calvaria. Mice were injected daily with vehicle alone (panel 1) or with simvastatin at 5 mg/kg/day (panel 2) or at 10 mg/kg/day (panel 3) for 5 days, and bones were then histologically examined at day 21. There is a marked increase in new bone width in statin-treated versus vehicle-treated mice. Panel 4 represents rhFGF-1 (12.5 μg/kg/day) and panel 5 represents rh-BMP-2 (30 μg/kg/day) as positive controls. They cause effects similar to those of simvastatin. The arrows indicate the new bone that has been formed as a consequence of simvastatin treatment. (B) Quantitative effects of simvastatin on the width of calvarial bones after local injection into the scalp in (A). Total bone area of the right calvaria (the injected side) was measured from the site of muscle implantation to the suture. The width of the new calvarial bone formed was measured at four points (at 0.5-mm intervals), starting at the muscle implantation site. The mean was calculated for each calvaria measured. The effects are compared with those of FGF-1. of PBS (pH 7.2)]. These were administered by intraperitoneal injection before killing on days −14 and −4, respectively. We measured bone volume, osteoid volume (in plastic-embedded sections), osteoblast surface, osteoclast surface, and osteoclast number (16). Recombinant human FGF-1 (rhFGF-1) (experiment 1) and synthetic human PTH (amino acids 1 through 34 from the NH2-terminal end) (experiment 3) were used as positive controls. The statins caused increases in trabecular bone volume of between 39 and 94% after treatment. This was clearly due to an anabolic (bone-forming) effect because there was a parallel increase in BFRs with the use of dynamic parameters (Table 2). There was a concomitant decrease in osteoclast numbers where these were also assessed. Recently, it has been shown that certain bisphosphonates, which are inhibitors of bone resorption and are widely used as therapy for osteoporosis, also act on the cholesterol biosynthesis pathway (17–19) by targeting enzymes more distal in the mevalonic acid pathway than HMG Co-A reductase. It has been postulated that these enzymes are required for prenylation of small proteins such as Rho and Ras and that interference

| Treatment | Simvastatin | Lovastatin | Statin |FIG. 3. (A) Effect of simvastatin on new bone formation after local subcutaneous injection over the murine calvaria. Mice were injected daily with vehicle alone (panel 1) or with simvastatin at 5 mg/kg/day (panel 2) or at 10 mg/kg/day (panel 3) for 5 days, and bones were then histologically examined at day 21. There is a marked increase in new bone width in statin-treated versus vehicle-treated mice. Panel 4 represents rhFGF-1 (12.5 μg/kg/day) and panel 5 represents rh-BMP-2 (30 μg/kg/day) as positive controls. They cause effects similar to those of simvastatin. The arrows indicate the new bone that has been formed as a consequence of simvastatin treatment. (B) Quantitative effects of simvastatin on the width of calvarial bones after local injection into the scalp in (A). Total bone area of the right calvaria (the injected side) was measured from the site of muscle implantation to the suture. The width of the new calvarial bone formed was measured at four points (0.5-mm intervals), starting at the muscle implantation site. The mean was calculated for each calvaria measured. The effects are compared with those of FGF-1. of PBS (pH 7.2)]. These were administered by intraperitoneal injection before killing on days −14 and −4, respectively. We measured bone volume, osteoid volume (in plastic-embedded sections), osteoblast surface, osteoclast surface, and osteoclast number (16). Recombinant human FGF-1 (rhFGF-1) (experiment 1) and synthetic human PTH (amino acids 1 through 34 from the NH2-terminal end) (experiment 3) were used as positive controls. The statins caused increases in trabecular bone volume of between 39 and 94% after treatment. This was clearly due to an anabolic (bone-forming) effect because there was a parallel increase in BFRs with the use of dynamic parameters (Table 2). There was a concomitant decrease in osteoclast numbers where these were also assessed. Recently, it has been shown that certain bisphosphonates, which are inhibitors of bone resorption and are widely used as therapy for osteoporosis, also act on the cholesterol biosynthesis pathway (17–19) by targeting enzymes more distal in the mevalonic acid pathway than HMG Co-A reductase. It has been postulated that these enzymes are required for prenylation of small proteins such as Rho and Ras and that interference

**Table 1.** Effects of simvastatin (experiment 1) and lovastatin (experiment 2) on neonatal murine calvarial bone organ cultures. Explanted bones were cultured for 72 hours, and four bones were in each treatment group. Values are means ± SEM.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>New bone area (per mm² × 10⁻³)</th>
<th>Cells (per 0.3 mm of bone)</th>
<th>New bone area (per mm² × 10⁻³)</th>
<th>Cells (per 0.3 mm of bone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.4 ± 0.8</td>
<td>98 ± 7</td>
<td>4.5 ± 0.5</td>
<td>84 ± 3</td>
</tr>
<tr>
<td>BMP-2 (40 ng/ml)</td>
<td>6.5 ± 0.8*</td>
<td>145 ± 5*</td>
<td>7.9 ± 0.6*</td>
<td>110 ± 3*</td>
</tr>
<tr>
<td>Statin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.062 μM</td>
<td>5.3 ± 1.1</td>
<td>110 ± 9</td>
<td>4.4 ± 0.7</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>0.125 μM</td>
<td>8.2 ± 0.7*</td>
<td>135 ± 4*</td>
<td>7.6 ± 1.0</td>
<td>87 ± 13</td>
</tr>
<tr>
<td>0.25 μM</td>
<td>14.5 ± 1.8*</td>
<td>167 ± 17*</td>
<td>10.1 ± 0.6*</td>
<td>102 ± 5*</td>
</tr>
<tr>
<td>0.5 μM</td>
<td>14.0 ± 1.1*</td>
<td>190 ± 26*</td>
<td>12.4 ± 2.6*</td>
<td>132 ± 7*</td>
</tr>
</tbody>
</table>

*Significantly greater than control (P < 0.01).
Table 2. Effects of simvastatin on trabecular bone volume and bone formation rates. Simvastatin was given in doses of 5 to 50 mg/kg/day by oral gavage for 35 days to (i) 3-month-old virgin female rats (experiment 1), (ii) 3-month-old virgin female rats that had been ovariectomized within 7 days after the start of treatment (experiment 2), and (iii) 3-month-old virgin female rats that had been ovariectomized 2 months before treatment (experiment 3). In each treatment, the rats were weight matched and divided into treatment groups of 10. The rats were lightly anesthetized with isoflurane before ovariectomy. Animals were pair fed throughout the experimental period and body weights were determined weekly. Values in parentheses are percent change from vehicle-treated controls. BV/TV, bone volume/tissue volume; Ocl, osteoclasts; BFR, bone formation rate; OVX/veh, ovariectomized rats treated with vehicle; hPTH, human PTH; ND, not determined.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trabecular bone volume (% BV/TV)</th>
<th>BFR (μm²/μm²/day)</th>
<th>No. of Ocl/mm² of bone surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.4 ± 1.4</td>
<td>13.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Simvastatin (10 mg/kg/day)</td>
<td>18.6 ± 1.4* (±39)</td>
<td>ND</td>
<td>11.6 ± 1.4 (−15)</td>
</tr>
<tr>
<td>hFGF-1 (100 μg/kg/day)</td>
<td>21.4 ± 1.7* (±60)</td>
<td>ND</td>
<td>7.5 ± 1.3* (−45)</td>
</tr>
<tr>
<td>O VX/veh</td>
<td>6.9 ± 0.8</td>
<td>0.6 ± 0.1</td>
<td>8 ± 0.2</td>
</tr>
<tr>
<td>Simvastatin (1 mg/kg/day)</td>
<td>8.6 ± 0.41* (±25)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Simvastatin (10 mg/kg/day)</td>
<td>13.4 ± 2* (±94)</td>
<td>1.2 ± 0.1 (100*)</td>
<td>7 ± 0.3 (−12.5)</td>
</tr>
<tr>
<td>O VX/veh</td>
<td>4.6 ± 0.58</td>
<td>0.151 ± 0.01</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Simvastatin (5 mg/kg/day)</td>
<td>9 ± 0.8* (±96)</td>
<td>0.196 ± 0.021* (30)</td>
<td>0.9 ± 0.1 (−25)</td>
</tr>
<tr>
<td>Simvastatin (10 mg/kg/day)</td>
<td>8.6 ± 0.93* (±87)</td>
<td>0.229 ± 0.034* (52)</td>
<td>0.78 ± 0.06* (−33)</td>
</tr>
<tr>
<td>hPTH (80 μg/kg/day)</td>
<td>20 ± 1.9* (±348)</td>
<td>0.228 ± 0.025* (51)</td>
<td>0.84 ± 0.15 (−30)</td>
</tr>
</tbody>
</table>

*Significantly greater than control (P < 0.01).

with this process may lead to osteoclast apoptosis and cessation of bone resorption (18, 20). We cannot exclude the possibility that the statins both inhibit bone resorption and promote bone growth, and we did observe a concomitant decrease in osteoclast numbers (20). However, this effect appeared minor in comparison to the effect on new bone formation and osteoblast maturation.

The statins used in our studies and currently on the market are not ideal for use as systemic bone-activation agents. These statins were selected for their capacity to lower serum cholesterol, which requires targeting to HMG Co-A reductase in hepatic cells. Thus, the concentration of statin in other tissues is much lower than in the liver. The most efficacious statins would be those that distribute themselves to the bone or bone marrow. A preliminary retrospective analysis of older women taking lipid-lowering agents suggests that statin use is accompanied by greater hip bone mineral density and lower risk of hip fractures (relative risk = 0.30) (21); however, the sample size (598 statin users) was too small to yield definitive information.

The most powerful anabolic agents for bone are the peptide growth factors intrinsic to the tissue. For example, systemically administered FGF-1 restores trabecular microarchitecture and increases bone volume (15). However, all of the peptide growth factors have disadvantages—they can be mitogenic to other bone cells and nonselective in their effects. In addition, the FGFs cause hypotension, which limits their potential use in elderly patients (22).

Our results suggest that statins, which are orally bioavailable and have been safely administered to patients for more than a decade, may exert further investigation as potential anabolic agents for bone. When the doses are extrapolated from humans to rats with respect to lipid lowering, the statins’ effects on bone occur at doses similar to the lipid-lowering doses used in humans.

References and Notes
7. L. R. Garrett and G. R. Mundy, data not shown.
23. We thank L. Kneiruem, L. A. Trafford, and N. Garrett for the preparation of this manuscript.

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Requirement for B Cell Linker Protein (BLNK) in B Cell Development

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Linker proteins function as molecular scaffolds to localize enzymes with substrates. In B cells, B cell linker protein (BLNK) links the B cell receptor (BCR)-activated Syk kinase to the phosphoinositide and mitogen-activated kinase pathways. To examine the in vivo role of BLNK, mice deficient in BLNK were generated. B cell development in BLNK−/− mice was blocked at the transition from B220+ CD43+ precursor B to B220− CD43− precursor B cells. Only a small percentage of immunoglobulin M (IgM)− IgM+ B cell precursors were detected in the periphery. Hence, BLNK is an essential component of BCR signaling pathways and is required to promote B cell development.

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are required for the generation of second messengers (1). In turn, the coordinate generation of second messengers is important for normal B cell function because disruption of selected signaling pathways is associated with B cell anergy (2). Linker or adapter molecules play integral roles in linking the BCR-activated kinases with these enzymes. One such linker molecule, BLNK (also known as SLP-65, BASH, and BCA), is phos-