Cell signalling and bone remodelling
Part II: Developments in the pathogenesis and principles of management of selected skeletal disease states

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Abstract
Mapping of the bone remodelling signalling pathways contributed significantly to the establishment of a scientific basis for the development of pharmaceuticals which have the potential to induce or suppress bone formation. Enhancing bone healing and the establishment of a pre-determined skeletal phenotype are now within reach of the medical profession. This manuscript provides practitioners with an overview of recent developments in the quest for uncovering the molecular mechanisms involved in the pathogenesis of selected bone disease states and the role these discoveries play in the future management of bone healing and skeletal health.

Key words: bone remodelling, osteoporosis, metastatic bone disease, Paget’s disease, osteopetrosis.

Introduction
The study of rare genetic skeletal diseases exposed a wealth of information on the autocrine, paracrine and endocrine control of bone metabolism. Mapping of the pathways which control bone remodelling (covered in Part I) provides exciting new possibilities in the prevention and management of common skeletal deficiency states like osteoporosis. The field of regenerative medicine is growing rapidly and it is therefore no surprise that the pharmaceutical industry is investing large sums of money in the development of patented drugs and auto-antibodies which modulate the establishment of a specific skeletal outcome. Gene delivery to bone with viral vectors, plasmids or mesenchymal stem cells is certain to develop as potent tools in the manipulation of bone and treatment of skeletal disease.

Mesenchymal bone marrow stem cells have the potential to form bone, are chemically attracted to the skeleton after peripheral administration and are therefore ideal vehicles to deliver transgenes which induce anabolic or block catabolic cytokines. Reconstruction of large bone segments without cortico-cancellous bone grafts, which comes with the risk of disease transmission, infection and rejection, remains the prime goal of researchers in the field of tissue engineering. Using osteoconductive matrices seeded with osteogenic progenitor cells and osteoinductive and vasoproliferative factors to achieve this goal is no longer unrealistic from a scientific point of view. This article is aimed at providing practitioners with insight into recent advances on the impact of modulation of cell signalling on the management of selected skeletal disease states.
Osteoporosis

The persistent loss of bone with age ultimately culminates in osteoporosis. Although the fracture risk of young males is higher than females, in older populations significantly more women are affected than men. The societal consequences of osteoporosis are devastating and the cost implications of the more than 2 million fractures recorded in the USA in 2005 are projected to rise to $25 billion by 2025. Commercial interest in developing patented drugs that target this disease is flourishing. The most popular medication used in the prevention of osteoporosis are drugs in the bisphosphonate group, which can be administered orally, and directly inhibit osteoclasts and indirectly decrease osteoblast activity, thereby down-regulating bone metabolism. Denosumab, a monoclonal human antibody directed against RANKL that prevents osteoclastogenesis and bone catabolism, was approved in 2010 by the FDA (US Food and Drug Administration) for use in postmenopausal women at risk for osteoporosis. Parathyroid hormone (PTH) administration is the only FDA-approved anabolic therapy for fracture prevention in postmenopausal women. CD8+ T-cells produce Wnt ligand under intermittent stimulation by PTH. This activates the canonical Wnt signalling pathway in pre-osteoblasts and suppresses the production of sclerostin by osteocytes, facilitating osteoblast differentiation and bone formation (readers are referred to Part I* for more information on cell signalling involved in bone remodelling). The activation or inactivation of steps in Wnt signalling in osteoblasts can induce bone anabolism or – catabolism. Stimulation of bone formation can be achieved through auto antibodies directed against endogenous Wnt-antagonists such as Dickkopf-1 and sclerostin. Inhibition of cytoplasmic kinases involved in Wnt signalling by lithium stimulates bone formation. Vascular endothelial growth factor (VEGF) improves vascularisation and facilitates bone formation. Suspending the osteoblast-suppressing property of serotonin with drugs which antagonise its action may be the key to the development of a novel approach in preventing osteoporotic fractures. Although innovative research on laboratory animals shows promising results in many of these fields of research, interference with these pathways runs the risk of long-term secondary complications such as the induction of tumours. The ultimate goal is to manipulate steps in Wnt signalling which is bone-specific, thereby negating the development of unintended secondary pathology.

Inflammation

In the past decade the accumulation of data on the influence of inflammation on the skeleton has led to the development of a dedicated field of study referred to as ‘osteoimmunology’. Monocytes are attracted to a site of inflammation and induced to differentiate into macrophages which have the capacity to elaborate the osteoclastogenic nuclear factor κB (NF-κB) ligand, RANKL. Although several factors released during inflammation promote osteoclast activation, RANKL and its inhibitor, osteoprotegerin (OPG), are the final downstream cytokines that control osteoclast differentiation and bone resorption (see Part I*). The SOST gene which encodes for sclerostin is the only part of the Wnt pathway expressed exclusively by osteocytes and a monoclonal antibody, which inactivates SOST, is promising as interference in other cell processes appears to be limited. Inflammatory-associated bone loss not only occurs in the area of inflammation, but also through osteoclastogenic cytokines released in circulation by distant inflammations, such as rheumatoid arthritis. Resolution of a site of inflammatory-induced bone loss follows upon the elaboration of cytokines, such as proteins belonging to the transforming growth factor beta (TGF β) family, which stimulate bone formation. Human recombinant BMP7 (available commercially under the brand name OP1) is used to facilitate fusion of vertebrae to prevent neurological trauma. rhBMP2 is however more widely used to treat non-union of fractures as it appears to be superior in inducing new bone formation than the other BMPs. BMP7 has a potential future role in the management of chronic kidney disease through its inhibition of fibrosis and restoration of healthy epithelial cell populations. Mineralisation of the newly formed bone is mediated by bone sialoprotein, carboxylated osteocalcin or other cytokines (see Part I*). Mapping of these pathways exposed specific receptor binding sites on bone cells which can potentially be activated or blocked in order to either limit bone resorption or accelerate bone formation and mineralisation, impacting directly on the process of bone healing. Carrier systems which deliver bioactive molecules locally, such as the biocoating of implant surfaces with bone morphogenetic protein (BMP) (a protein of the TGF β family) and other osteogenic cytokines can facilitate integrative bone repair. Ceramic microsphere carriers are injectable, biodegradable and can be coated to become osteoinductive thereby decreasing the post-infection healing time of bone.

HIV and antiretroviral therapy

Antiretroviral therapy has changed the fate of HIV infection from a fatal to a manageable chronic disease. With this advancement the co-morbidities resulting from skeletal catabolism are now more prominent in this cohort of patients than in the past. Highly active antiretroviral therapy (HAART), chronic inflammation, the virus itself and dietary factors contribute to bone loss and the increase in the incidence of fractures reported in AIDS patients. A contributing factor is hypovitaminosis D which is prevalent among HIV-positive subjects. Although the mechanisms involved in the skeletal anabolism of HIV patients on HAART are not fully understood, data now indicate that these patients should be
included in screening programmes as high risk for osteoporosis. Recent focus on the prominent role the immune system plays in skeletal health makes the influence of the residual immune dysregulation syndrome in treated HIV patients an unexploited field for research.

**Generalised bone forming diseases**

Osteopetrosis is a heterogenous disease with several molecular and genetic defects leading to dysfunctional osteoclasts and unopposed bone formation. Among others, mutations of M-CSF and RANK are involved, as well as over-expression of OPG, as the RANKL/OPG ratio is a major determinant of bone mass. The clinical severity varies from neonatal onset with bone marrow displacement and fatal pancytopenia to an incidental finding of bone sclerosis on a radiograph. Repopulation of the bone marrow with normal stem cell populations provides some hope for patients suffering the infant-onset types. The duplication of the signalling peptide (TNFRSF11A) of the gene that encodes for RANK, is associated with a rare panostotic expansile bone disease (distinguished from fibrous dysplasia by an absence of GNAS mutation). Similar RANK insertion mutations were reported in other expansile bone conditions. Sclerosing bone dysplasias (sclerosteosis, Worth syndrome and Van Buchem disease) are linked to a genetic mutation which either incapacitates osteocytes to produce sclerostin or modulates LRP5 or its receptors. Simulation of these mutations through gene transferral may form a basis for the development of therapeutic agents that facilitate bone formation and improve bone healing after surgical procedures.

**Paget’s disease of bone**

Although a decline in the incidence has been reported in several communities in which Paget’s disease is endemic, it remains an important diagnosis in orthopaedic practice. In the advanced stage, it is earmarked by disordered bone formation which leads to skeletal deformity, pathological fractures and neurologic pains and deafness due to compression of nerves which pass through the enlarging bony structures. Paget’s disease is the result of a combination of a genetic mutation in the SQSTMI/p62 gene and the impact of an environmental factor, most likely chronic interleukins and RANKL. The bisphosphonate group of drugs (which block the effects of PTHrP) and denosumab are effective in addressing the skeletal morbidity and hypercalcaemia resulting from the production of osteoclastogenic cytokines by metastatic malignant clones in bone. Inactivation of osteoclasts is however not without complications as patients may develop osteonecrosis (particularly of the jaws), especially those on intravenous nitrogen containing bisphosphonates. Transforming growth factor β (TGF β) is released from the matrix of bone during resorption and stimulates the elaboration of several catabolic cytokines by tumour cells. The blocking of TGF β production in breast cancer through the therapeutic administration of SD-208, an inhibitor of TGF β, may therefore decrease the skeletal morbidity of breast cancer patients. Prostate cancer metastasis dysregulates bone remodelling and the nett outcome is bone formation, often described as an ‘osteoblastic’ response. The neoplastic cells produce growth factors such as insulin-like growth factor, platelet-derived growth factor, adrenomedullin and a recently discovered vasoactive peptide ET-1. The mechanism of stimulation of osteoblasts by ET-1 is unclear. Blocking of the endothelin A receptor (ET AR) by atrasentan, an ET AR antagonist, reduces skeletal morbidity in patients with advanced prostate cancer. This discovery in a rapidly advancing field of research is likely to introduce a new chapter in the management of this disease.

**Malignant disease**

Manipulation of the bone microenvironment is a field in which the next thrust in anticancer therapy is predicted. The influence of metastatic deposits of solid malignancies on bone is resorption, bone formation or both. Breast cancer is the prototype of the bone-resorbing and prostate cancer of the bone-forming phenotype. Factors produced by malignant deposits that stimulate osteoclasts include parathyroid hormone related protein (PTHrP), several of the interleukins and RANKL. The bisphosphonate group of drugs (which block the effects of PTHrP) and denosumab are effective in addressing the skeletal morbidity and hypercalcaemia resulting from the production of osteoclastogenic cytokines by metastatic malignant clones in bone.

**Conclusion**

The study of bone has moved beyond morphology, and exciting fields of research have been uncoverd in the last decade. Cell signalling pathways can now be linked to specific disease states and, through intervention, a specific bone phenotype can be induced by cytokine modulation. More studies are, however, required as the long-term effects of interfering in skeletal metabolism, which is part of systemic metabolic pathways, are as yet unknown.

**Conflict of interest statement**

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