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MEETING REPORT

Mechanistic and therapeutic insights into skeletal biology learned from the study of rare bone diseases

Laura L Tosi[1], Patricia Fonseca[2] and Matthew Warman[3]

[1]Division of Orthopaedics and Sports Medicine, Children's National Health System, Washington DC, USA. [2]Freelance Medical Writer, London, UK. [3]Orthopaedic Research Laboratories, Boston Children's Hospital, Boston, MA, USA.

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Meeting Report from the National Bone Health Alliance/Rare Bone Disease Patient Network Workshop, Houston, TX, USA, 11 September 2014

Introduction

To date, nearly 500 unique rare bone diseases have been described, which in the aggregate affect 1 in 5000 live births and comprise 5% of all birth defects. Currently, the overwhelming majority of these disorders have no cure. Studying rare bone diseases has helped elucidate biological pathways and reveal valuable therapeutic targets that also benefit patients with common bone diseases.

In an effort to engage scientists, clinicians, young investigators, advocacy groups and representatives of the pharmaceutical industry in fruitful discussion of how the study and treatment of rare bone diseases has helped, and will continue to help, expand our understanding of bone health, The National Bone Health Alliance and Rare Bone Disease Patient Network sponsored this workshop in partnership with the American Society for Bone and Mineral Research and the United States Bone and Joint Initiative.

The nosology of rare bone diseases

Deborah Krakow (UCLA, Los Angeles, CA, USA) opened the workshop with a review of the nosology of heritable skeletal disorders. Early efforts to classify this heterogeneous group of diseases were primarily based on radiographic and clinical findings. Significant phenotypic overlap between some disorders creates the need for a thorough classification system to facilitate what can become a challenging diagnosis. The latest revision of the *Nosology and Classification of Genetic Skeletal Disorders* combines pathological, histologic and developmental observations, as well as biochemical and molecular aspects.[1] In the age of molecular medicine, evolving disease classification schemes contribute to improved diagnosis, enabling researchers to delineate novel disorders and define allelic series that elucidate normal and diseased bone physiology.

Osteoblasts and osteocytes

Lynda Bonewald (University of Missouri, Kansas City, MO, USA) discussed how the study of rare bone disorders affecting osteoblasts and osteocytes has provided valuable insights into bone homeostasis. Deleterious mutations in osteoblast-modulating factor sclerostin, produced by osteocytes, result in sclerosing bone syndromes. By contrast, activating fibroblast growth factor 23 (FGF23), also expressed in osteocytes, causes hypophosphatemic rickets.[1] The latter pathology can potentially be counteracted through the use of FGF23 blocking antibodies, supporting the use of agents targeting specifically the osteocyte population.

Osteoclasts

Stuart Ralston (University of Edinburgh, UK) provided an overview of how studies in osteopetrosis and Paget disease elucidated osteoclast biology by uncovering molecules that regulate osteoclast differentiation and function. Some of the new drugs used to treat common bone diseases target molecules implicated in osteoclast pathologies; these include denosumab, an antibody against the receptor activator of nuclear factor kappa-B ligand (RANKL), and odanacatib, an inhibitor of cathepsin K.[2] Important future directions would be to exploit the use of genetic markers for early diagnosis and therapeutic intervention and to develop tailored treatments for osteoclast diseases.

Chondrocytes

Maurizio Pacifici (Children's Hospital of Philadelphia, Philadelphia, PA, USA) discussed rare diseases affecting the growth plate that have elicited new insights into normal skeletal growth and morphogenesis. The pediatric disorder hereditary multiple exostoses is characterized by the formation of benign cartilaginous tumors (exostoses) next to the growth plate and is caused by loss-of-function mutations in glycosyl synthases

EXT1 and EXT2, resulting in systemic heparan sulfate deficiency. These molecules assist in maintaining the function of the growth-plate–perichondrial border;[3] exostoses arise as a result of defects in this border function. In addition, mutations in growth differentiation factor 5 have recently been identified in patients with specific forms of brachydactyly or multiple synostoses syndrome,[1] contributing significantly to the knowledge of how complex signaling networks regulate skeletal development and sustain joint formation and skeletal growth.

Vasculature

Bjorn Olsen (Harvard Medical School, MA, USA) reviewed the role of the dynamic bone–blood tissue boundary. Osteoblast differentiation is intertwined with vascular development, and abnormalities in angiogenesis can cause significant skeletal malformations.[4] Pathologies intersecting blood and bone homeostasis reflect the cross talk between signaling pathways essential for cellular differentiation and maintenance processes shared by both systems. Rare bone diseases such as hamartoma, congenital lipomatous overgrowth, vascular malformations, epidermal nevi and spinal/skeletal anomalies/scoliosis (CLOVES), Klippel–Trenaunay and Proteus syndromes are caused by mutations in proteins that participate in vascular endothelial growth factor signaling,[5] which maintains vascular, skeletal and extracellular matrix homeostasis, whereas signaling abnormalities affecting lymphangiogenesis are associated with Gorham–Stout disease and growth retardation, alopecia, pseudoanodontia and the progressive optic atrophy (GAPO) syndrome.

The matrix

The primary structural component of the extracellular matrix, type I collagen, has long been implicated in osteogenesis imperfecta (OI). Brendan Lee (Baylor College of Medicine, Houston, TX, USA) reviewed recent data showing that the underlying deficiencies in this heterogeneous group of diseases are not merely structural. Recessive forms of OI comprise defects in enzymes responsible for collagen post-translational modifications, such as the prolyl 3-hydroxylase-1 (P3H1) complex. Complete ablation of cartilage-associated protein (CRTAP), a component of the P3H1 enzymatic complex, stimulates transforming growth factor-beta signaling.[6] Animal studies combined with genetic analysis of patients support that alterations in collagen biochemistry can have a strong impact on its folding, intracellular trafficking, extracellular assembly and cross-linking and lead to abnormal communication between the matrix and the bone cell reservoirs.

The transcriptional landscape in the skeleton

Chondrogenesis involves the concerted action of master regulatory signals at different steps. Whereas RUNX2 is essential to switch cells into hypertrophic differentiation programs at an early stage, sex-determining region Y-box 9 (SOX9) is essential for chondrocyte differentiation.[7] Andrew McMahon (University of Southern California, Los Angeles, CA, USA) showed that SOX9 acts as a focal point in the chondrocytic gene regulatory network, binding to more than 20 000 regions in the genome. These regions, associated with transcriptional regulators and chromatin-modifying enzymes, enable the coordinated (in)activation of genes involved in differentiation and proliferation, thus ensuring a harmonious growth of the skeleton.

Treating osteoclastic overactivity

Treatment of low bone mass disorders has traditionally targeted overactive osteoclasts. Graham Russell (University of Oxford, UK) compared the effects of bisphosphonates and those of denosumab on these cells. Bisphosphonates are widely available for the treatment of osteoporosis and Paget disease and in the management of bone complications in cancer. Denosumab shows equivalent efficacy in the treatment of osteoporosis but differs in patterns of reversal of resorption inhibition and duration of effect.[8] Despite these significant advances, patients would greatly benefit from an expanded armamentarium against bone resorption disorders.

Targeting enzymes and other proteins to bone

Low bone density can also derive from defects in mineralization. Loss-of-function mutations in tissue nonspecific alkaline phosphatase (TNSALP), a cell-surface enzyme with an active role in bone mineralization, are responsible for rare hypophosphatasia (HPP),[1] for which there is currently no effective treatment. Michael Whyte (Washington University, St Louis, MO, USA) presented positive results for asfotase alfa—a recombinant protein containing the TNSALP homodimer and a terminal deca-aspartate motif that directs the agent to bone tissue—in the treatment of infantile- and juvenile-onset HPP in several phase II clinical studies, supporting the use of targeted enzyme replacement therapy in the context of bone diseases.

Antibody-based modulation of extracellular signaling

Matthew Warman (Boston Children's Hospital, Boston, MA, USA) described the historic use of antibodies to treat human disease and presented results from animal studies supporting the use of antibodies targeting wingless-related integration site (Wnt) signaling to improve bone mass and strength. Certain missense mutations in low-density lipoprotein receptor-related protein 5 (LRP5) make LRP5 resistant to sclerostin, and by doing so increase bone strength. Proof-of-concept genetic studies were carried out to determine whether modulating Wnt signaling would benefit patients with rare skeletal fragility diseases, such as OI, by crossing Lrp5 high bone mass-causing knockin mutations in mouse models of OI.[9] In addition, these models were treated with sclerostin-neutralizing antibody.[9] Both approaches resulted in improved bone properties, offering new therapeutic strategies for patients with OI.

Inhibition of intracellular signaling by small molecules

The mammalian target of rapamycin (mTOR)/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as AKT) pathway combines intracellular signals to regulate cell growth, proliferation and survival.[10] Denise Adams (Cincinnati Children's Hospital, Cincinnati, OH, USA) summarized the latest advances in the use of small molecule inhibitors of PI3K/mTOR in the treatment of cancer. In preclinical studies, mTOR inhibitor

everolimus inhibited the proliferation of osteosarcoma cells and improved tumor response when used in combination with zoledronate.[11] A combination of everolimus and an aromatase inhibitor significantly improved survival in patients with metastatic breast cancer and reduced incidence of malignant progression in bone (BOLERO-2 study). Somatic mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) have been identified in several rare vascular anomalies with bone involvement such as lymphatic malformation and the Klippel–Trenaunay syndrome. Early studies suggest the efficacy of mTOR inhibitor sirolimus on the bone manifestations of these disorders and other disorders with lymphatic involvement.

Use of synthetic polypeptides to promote skeletal growth

The workshop closed with Laurence Legeai-Mallet (INSERM U1163, Imagine Institute, Paris, France), who presented the results of preclinical studies of a new C-type natriuretic peptide analog BMN-111 in the treatment of achondroplasia resulting from activating mutations in FGF receptor 3 (FGFR3). *In vitro*, BMN-111 counteracts constitutively active FGFR3 through inhibition of extracellular signal-regulated kinase-1/2 (ERK1/2) phosphorylation in primary chondrocytes obtained from patients and promotes proliferation and differentiation of murine chondrocytes heterozygous for the *Fgfr3* Y367C mutation *ex vivo*. Administration of BMN-111 to $Fgfr3^{Y367C/+}$ mice promotes growth of the axial and appendicular skeleton, with recovery of the natural architecture of the growth plate.[12] A phase II clinical trial in children with achondroplasia is ongoing.

Conclusion

The study of patients with rare diseases has provided extraordinary insights into processes by which the skeleton forms, grows and maintains itself throughout a lifetime of use. Genes with mutations leading to severe phenotypic manifestations identified in patients with rare diseases, via genome-wide association studies, are recognized to have milder variants that contribute to common disorders such as osteoporosis and osteoarthritis. Rare diseases have thus identified pathways that are being targeted to treat patients with rare and common disorders of the skeleton system. Despite these remarkable accomplishments, and the availability of a few satisfactory treatments for individuals with rare genetic diseases, much more work needs to be done. Also, to maximize financial returns on investments, patients whose rare diseases led to the identification of new approaches for treating both rare and common diseases often have to wait for new therapies to be first tested and approved in the patients with the common disorder, with considerable negative impact for their long-term management.

Conflict of Interest

PF is a freelancer medical writer and an editor for IMS Health. The remaining authors declare no conflict of interest.

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