PROSPECTS
Journal of Cellular Biochemistry 116:2139–2145 (2015)

Journal of Cellular Biochemistry

Genetic Approaches To Identifying Novel Osteoporosis Drug Targets

Robert Brommage*
Lexicon Pharmaceuticals, The Woodlands, Texas

ABSTRACT

During the past two decades effective drugs for treating osteoporosis have been developed, including anti-resorptives inhibiting bone resorption (estrogens, the SERM raloxifene, four bisphosphonates, RANKL inhibitor denosumab) and the anabolic bone forming daily injectable peptide teriparatide. Two potential drugs (odanacatib and romosozumab) are in late stage clinical development. The most pressing unmet need is for orally active anabolic drugs. This review describes the basic biological studies involved in developing these drugs, including the animal models employed for osteoporosis drug development. The genomics revolution continues to identify potential novel osteoporosis drug targets. Studies include human GWAS studies and identification of mutant genes in subjects having abnormal bone mass, mouse QTL and gene knockouts, and gene expression studies. Multiple lines of evidence indicate that Wnt signaling plays a major role in regulating bone formation and continued study of this complex pathway is likely to lead to key discoveries. In addition to the classic Wnt signaling targets DKK1 and sclerostin, LRP4, LRP5/LRP6, SFRP4, WNT16, and NOTUM can potentially be targeted to modulate Wnt signaling. Next-generation whole genome and exome sequencing, RNA-sequencing and CRISPR/CAS9 gene editing are new experimental techniques contributing to understanding the genome. The International Knockout Mouse Consortium efforts to knockout and phenotype all mouse genes are poised to accelerate. Accumulating knowledge will focus attention on readily accessible databases *(Big Data)*. Efforts are underway by the International Bone and Mineral Society to develop an annotated *Skeletome* database providing information on all genes directly influencing bone mass, architecture, mineralization or strength. J. Cell. Biochem. 116: 2139–2145, 2015.

KEY WORDS: OSTEOPOROSIS; DRUG DEVELOPMENT; SKELETOME; GENOMICS

PRESENT AND POTENTIAL NEAR-TERM OSTEOPOROSIS THERAPIES

Existing drugs to treat osteoporosis [Das, Ferrari, 2014] target estrogen receptors (various estrogens and raloxifene), farnesyl diphosphate synthase (four amino bisphosphonates), the Rank Ligand receptor (denosumab), the parathyroid hormone receptor (teriparatide) and strontium ranelate (in the EU only). Odanacatib (cathepsin K inhibitor) has completed Phase 3 clinical trials and Phase 3 trials for romosozumab (anti-sclerostin antibody) are underway. The anti-resorptive therapies (estrogens, raloxifene bisphosphonates, denosumab, and odanacatib), having various dosing regimens, all reduce vertebral fractures by about 50% and there is minimal motivation for developing additional drugs in this class.

The anabolic therapeutic teriparatide is given by daily subcutaneous injections for two years, after which patients are usually switched to one of the anti-resorptive therapies to prevent loss of the bone gaining during teriparatide treatment. Several studies examining weekly rather than daily dosing showed reduced efficacy. Considerable efforts have been made to develop alternate routes of administration for teriparatide analogues (oral, buccal, inhalation), with oral dosing showing some success [Augustine and Horwitz, 2013]. The PTHrP analogue alaboparatide [Leder et al., 2015] completed Phase 3 clinical trials examining subcutaneous administration. Romosozumab, given by monthly subcutaneous injections for one year, was effective in Phase 2 trials [McClung et al., 2014] and is undergoing Phase 3 clinical trials. A major unmet need in osteoporosis therapy is for an orally active anabolic agent.

Drug candidates undergoing or having completed Phase 2 clinical trials include alaboparatide administered by a transdermal skin patch and the c-Src inhibitor saracatinib. Potential osteoporosis therapies that failed during clinical trials include a non-amino bisphosphonate (tiludronate), SERMs (arzofoxifene, bazedoxifene, idoxifene, lasofoxifene, levormeloxifene), calcium-sensing receptor agonists (ronacaleret, JTT-305/MK-5442, AXT914), tibolone (targeting multiple sex hormone receptors) and cathepsin K inhibitors

*Correspondence to: Institute of Experimental Genetics, German Mouse Clinic, Helmholtz Center Munich, 85764 Neuherberg, Germany.
E-mail: robert.brommage@helmholtz-muenchen.de
Manuscript Received: 23 March 2015; Manuscript Accepted: 30 March 2015
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 1 April 2015
DOI 10.1002/jcb.25179 • © 2015 Wiley Periodicals, Inc.

(balicatib, relacatib). EU approval for full length PTH [1–84] (Preotact) has been withdrawn.

BONE REMODELING VERSUS BONE MODELING

Bone modeling includes the processes by which bone is formed during growth. Whereas juvenile osteoporosis typically involves low bone formation, postmenopausal, aging and glucocorticoid-induced osteoporosis all result from bone formation failing to match resorption during remodeling (turnover) cycles. Osteoblasts and osteoclasts communicate using multiple signals and this signaling is disrupted in osteoporotic bone. Anti-resorptive therapies work by inhibiting osteoclast bone resorbing activity and efficacy is readily monitored by reductions in serum biomarkers of bone resorption. During the early 1980s hypotheses about designing strategies to stimulate bone formation focused on activating bone remodeling to initially stimulate osteoclast differentiation, blocking the subsequent bone resorption but then allowing the osteoblastic, bone-forming portion of the remodeling cycle to proceed.

Teriparatide treatment activates bone remodeling on trabecular bone surfaces but also within osteonal cortical bone. But a key component of the anabolic actions of teriparatide involves inducing new bone formation on quiescent bone surfaces (bone modeling) by increasing osteoblast formation and also activating bone-lining cells (quiescent osteoblasts) to form new bone. Romosozumab treatment also stimulates bone modeling on quiescent surfaces without inducing bone remodeling [Ominsky et al., 2014].

THE BONE MECHANOSTAT

Why does the capacity to activate bone modeling, employed during growth, exist in adults? The bone mechanostat theory provides a reasonable explanation for this apparent dilemma. Full discussion of this theory is beyond the scope of this review. Briefly, as a structural tissue providing strength, bone architecture adapts to perceived mechanical forces. When mechanical stress is low bone is lost but when forces are high bone responds by activating modeling processes to increase its strength. Teriparatide and anti-sclerostin treatments stimulate downstream factors involved in mechanostat sensing and action in ways that mimic the responses to high mechanical stress. Because the mechanostat pays a key role in determining bone mass, additional drug targets are likely to be identified as our understanding of this feedback system increases [Kang and Robling, 2015].

There are probably several, likely redundant, biochemical pathways involved in the mechanostat. Theories universally propose that strain-induced fluid flow within bone canaliculi activates osteocyte mechanostat signals that ultimately promote bone modeling. PTHrP is produced by osteocytes and its expression is stimulated by stretch in tissues such as the mammary gland, urinary bladder and cultured osteoblast-like cells. One theory of mechanostat function posits that PTHrP secretion by osteocytes in response to canalicular fluid flow acts to both promote osteoblast WNT signaling and, in a paracrine fashion, inhibit osteocyte secretion of sclerostin, thereby activating bone modeling. Since PTHrP and teriparatide both signal through the PTH receptor, (intermittent) teriparatide treatment mimics pulsatile PTHrP secretion in response to skeletal loading. Sclerostin inhibits bone WNT signaling by binding to the WNT co-receptors LRP5 and LRP6 [Chang et al., 2014b] and this theory therefore successfully links the anabolic actions of two potent bone anabolic therapies with the known role of the WNT pathway on bone formation.

Numerous studies support this mechanostat theory, outlined in Figure 1. Osteocyte-specific KO of the PTH receptor blunts the anabolic effect of teriparatide treatment [Powell et al., 2011; Saini et al., 2013] whereas transgenic mice with osteocytes having a constitutively active PTH receptor show dramatic elevations in bone mass [O'Brien et al., 2008] if LRP5 is present [Rhee et al., 2011]. Bone formation in response to mechanical loading is reduced in *Lrp5* KO mice [Zhao et al., 2013] but enhanced in transgenic mice expressing activating *Lrp5* mutations [Niziolek et al., 2012]. A similarly poor responsiveness to skeletal loading is observed in *Wnt16* KO mice [Wergedal et al., 2015] and in mice expressing a constitutively active SOST gene [Tu et al., 2007].

Given that sclerostin inhibits WNT signaling, which of the 19 WNTs are physiologically active in bone must be resolved. Most canonical WNTs likely stimulate bone formation when provided exogenously but may not be normally present within bone. Thus, bone mass is dramatically elevated in transgenic mice over-expressing WNT10B but only modestly reduced in *Wnt10b* KO mice. Mutations in WNT1 produce skeletal defects in humans and mice. *Wnt16* KO mice have reduced cortical but normal trabecular bone mass [Movérare-Skrtic et al., 2014; Wegerdal et al., 2015] and human WNT16 polymorphisms influence BMD. Since bones from *Wnt16* KO mice do not respond to the bone-forming effects of mechanical loading, this WNT certainly is a major factor transducing mechanical stimulation to osteoblast activation [Wegerdal et al., 2015].

DRUG TARGETS

Contemporary rational drug design includes knowledge of drug targets—the body's molecules with which drugs interact. Historically, drug targets were often initially unknown as was the case during

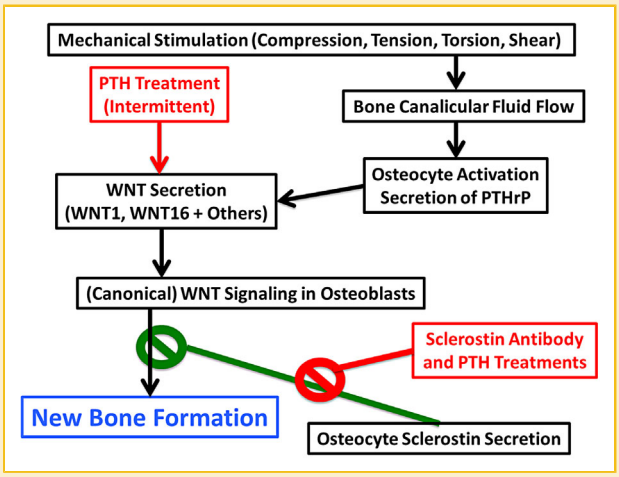

Fig.1. Proposed Mechanism for the Stimulation of Bone Formation following Mechanical Stimulation. Green represents the physiological inhibition of bone formation by sclerostin secreted by osteocytes. Red indicates pharmacological interventions that accentuate physiological mechanisms involved in the mechanostat response. See text for further discussion.

bisphosphonate development. Bisphosphonates were optimized employing rational drug design by examining bifunctional molecules having a hydroxyapatite binding component linked to an osteoclast poison. The molecular target of the poison component, farnesyl diphosphate synthase, was identified several years after the approval of alendronate in 1985. Understanding a drug's molecular mechanism of action is not required for regulatory approval.

By examining commonalities of approved drugs, secreted proteins and cell surface receptors are targets for antibodies with enzymes, receptors, transporters and channels being targets for small molecules [Sakharkar and Sakharkar, 2007]. Structural proteins and transcription factors are presently extremely difficult to modulate therapeutically, as can be appreciated by attempting to imagine an approach to modify mutant collagens causing osteoporosis imperfecta. Selecting new drug targets engenders much discussion [Swinney, 2004; Spector and Veselle, 2006] and increasing knowledge of genome has yielded identification of most transporters and channels. Undoubtedly, additional enzymes yielded potential drug targets remain to be discovered [Overington et al., 2006].

THE GENOMICS REVOLUTION

The *Skeletome* is proposed as a new term to include all genes that directly influence skeletal mass, architecture, mineralization or strength. Progress in translational medicine from the ongoing genomics revolution continues at a rapid pace and accumulating knowledge will be employed to evaluate novel drug targets for osteoporosis. Contributions from genomics towards existing treatments include 1) RNA expression studies leading to the discovery of high levels of cathepsin K in osteoclasts; 2) transgenic mice overexpressing osteoprotegerin leading to the discovery of the RANKL receptor; and 3) identification of SOST gene mutations in patients with osteosclerosis leading to the discovery of sclerostin. Gene knockout mice with disruptions in *Ctsk*,*Tnsf11a* and *Sost* all show dramatic changes in bone mass consistent the drug effects observed by inhibiting the corresponding proteins.

As discussed in conjunction with the mechanostat, the WNT signaling pathway plays a key role in bone formation and resorption and likely mediates the anabolic skeletal actions of teriparatide [Baron and Kneissel, 2013; Maupin et al., 2013]. Antibodies to sclerostin are in clinical trials and an antibody to DDK1 is being evaluated for multiple myeloma [Zhou et al., 2013]. Potential osteoporosis drug targets in the WNT pathway include WNT16 (Movérare-Skrtic, Wergedal), SFRP4 (Brommage) and LRP4. *Lrp4* KO mice have high bone mass and treating mature rats with neutralizing antibodies against LRP4 increases cortical and trabecular bone mass [Chang et al., 2014a].

DISCOVERING NOVEL GENES AFFECTING THE HUMAN SKELETON

Identifying mutated genes responsible for spontaneous human mutations has provided important insights, including identifying the critical functions of sclerostin (osteosclerosis and van Buchem's disease), LRP5 (High Bone Mass and Osteoporosis Pseudoglioma Syndromes), LRP6 [Mani et al., 2007] and cathepsin K (pycnodysostosis). Analysis of bone cells from HBM patients having activating LRP5 mutations showed the mutated protein was unresponsive to the inhibiting actions of DKK1 [Boyden et al., 2002]. Many osteopetrosis genes affecting osteoclast formation and activity have been identified [Aggarwal, 2013; Sobacchi et al., 2013]. Comprehensive Online Mendelian Inheritance in Man (OMIM) and International Skeletal Dysplasia Society [Warman et al., 2011] databases are continually updated with the latest discoveries. Development of next generation sequencing technologies to sequence whole exomes and whole genomes has revolutionized this field and continued advances are expected [Gregson et al., 2012; Brunham and Hayden, 2013; Farber and Clemens, 2013; Sule et al., 2013].

Human Genome-Wide Association Studies (GWAS) have identified 100+ genes having polymorphisms affecting bone mass. A full review of these studies is beyond the scope of this review, as this topic has been reviewed [Hsu and Kiel, 2012; Urano and Inoue, 2014]. Except for studies in specific isolated populations, the GWAS era utilizing DEXA BMD measurements has likely reached its natural limitations. However, bone mass measurement at various skeletal sites by 3D CT rather than 2D DEXA can provide architectural information unavailable y DEXA.

CANDIDATE GENES IN MICE

Mario R. Capecchi, Martin J. Evans and Oliver Smithies were awarded the 2007 Nobel Prize in Physiology or Medicine for their groundbreaking work developing methods to inactivate (knockout) any desired gene in mice. Individual investigators, selecting candidate genes for analyses, have generated thousands of knockout mice and identified numerous genes affecting the *Skeletome*. In addition to expected phenotypes, genes predicted to affect bone have been observed to be unimportant and genes not previously known to affect bone (such as *Src*) have shown dramatic skeletal phenotypes. Candidate gene studies continue in laboratories throughout the world. Recent development of the CRISPR/CAS9 gene editing technology is expected to greatly reduce times required to generate KO and point mutations mice.

INTERNATIONAL KNOCKOUT MOUSE CONSORTIUM (IKMC)

Although the candidate gene approach has been fruitful, it has limitations. Many candidate genes have now been examined and individual laboratories usually have expertise in phenotyping KO mice for their area of scientific interest. Thus, a neurology group is unlikely to look for skeletal phenotypes and bone groups may not seek non-skeletal phenotypes for genes affecting bone. The IKMC was formed as an international effort to KO and phenotype all 20,000+ protein-coding genes in mice. Comprehensive phenotyping is performed at centers and all data are uploaded to the International Mouse Phenotyping Consortium (IMPC) website, with currently over 1,400 gene KOs examined. Gene KOs identified to-date having dramatic skeletal phenotypes include *Cyp27b1* (vitamin D-1-hydroxylase), *Kiss1r* (hypothalamic receptor initiating puberty), *Lrrk1* (osteopetrosis), *Phex* (hypophosphatemic rickets) and *Wnt16* (spontaneous fractures). Suggestive body BMD phenotypes (in the primary DEXA screen) have been observed for novel genes coding for a minimally studied cytochrome (*Cyp4b1*), a collagen-interacting protein (*Tram2*) and the sphingosine phosphate transporter (*Spsn2*). Human *PLS3* mutations cause X-linked juvenile osteoporosis and

low BMD was observed in *Pls3* KO mice. Separately from the IKMC, Lexicon Pharmaceuticals generated over 4,700 mouse KOs between 2000 and 2008 and the skeletal phenotypes observed have been published [Brommage, 2014].

The IMKC campaign is expected to accelerate over the next several years and efforts are underway through the International Bone and Mineral Society (IBMS) to develop an online database annotating and summarizing skeletal phenotypes from the IMPC website. As the IKMC progresses deeper into the genome, genes coding for proteins for which there is minimal knowledge of their actions will increasingly be examined. The challenge will be to determine functions and biochemical pathways for genes that, for example, code for hypothetical enzymes.

SPONTANEOUS MOUSE MUTATIONS AND MUTAGENESIS STUDIES

Spontaneous mutations [Davisson et al., 2007] in several genes produce osteopetrosis in mice and rats [van Wessenbeeck and Van Hul, 2005] and the *swaying* mouse has skeletal defects resulting from a *Wnt1* mutation [Joeng et al., 2014] . N-ethyl-N-nitrosourea (ENU) is a supermutagen producing random DNA single base pair mutations. ENU mutagenesis campaigns have identified mutations in genes affecting 1) skeletal patterning (*Ankrd11, Arsb, Ctnb1, Col2a1, Fbn2, Fgfr1, Flnb, Gdf5, Gja1, Hoxd12, Kif7, Lmbr1, Lrp6, Nell1, Npr3, Plzf, Trip11, Twist, Xylt1*); 2) bone mass (*Col1a1, Crh, Enpp1, Ostm1, Ptpn6, Rankl, Tcirg1, Zdhhc13*); and 3) mineral homeostasis (*Alpl, Asgr1, Casr, Galnt3, Gnas, Jak1, Phex, Trpv5, Umod*). Several laboratories have performed whole exome sequencing of DNA from ENU-treated mice to identify mutated genes and cryopreserved sperm from these mice are available. Advantages of ENU mutagenesis over gene knockout campaigns include the possibility of obtaining activating mutations and, as is usually the case with human mutations, single amino acids are affected rather than whole gene deletions.

RNA SEQUENCING (RNA-SEQ)

Massive parallel sequencing of mRNA is replacing gene chip technologies to fully characterize mRNA expression over a wide range of abundance with single nucleotide resolution. Non-coding RNAs can also be analyzed. Initial efforts in bone have included studies examining 1) bones from control mice, mice having an activating *Lrp5* mutation and *Lrp5* knockout mice [Ayturk et al., 2013]; and 2) an established osteocyte cell line treated separately with PTH and 1, 25-dihydroxyvitamin D [St John et al., 2015]. Future RNA-Seq studies examining bones from various patient populations, transgenic and knockout mice, and bone cell cultures will undoubtedly provide information on genes and molecular pathways regulating bone mass that are potentially targetable by drugs. Of particular interest are downstream pathways of PTH and sclerostin action involved in bone formation.

COMPARATIVE GENOMICS

Genes influencing skeletal biology have been remarkably well conserved during mammalian evolution and genes having distinct actions in mice and humans are exceedingly rare. One example is the *PLEKHM1* gene, for which two separate mouse KOs have normal bone mass whereas mutations in human and rat genes cause osteopetrosis. This consistency has been a major advantage in using preclinical models for osteoporosis drug development. One clear skeletal difference between rodents and primates is the lack of normal osteonal remodeling (turnover) in rodent cortical bone. Since cortical bone was experimentally easier to examine histologically than trabecular bone during the 1960s, rodents were initially believed to be poor models for human bone metabolism. As experimental techniques improved during the 1970s allowing histolomorphometric measurements of trabecular bone in ovariectomized rats (pioneered by Tom Wronski and Dike Kalu), rats and mice became the standard species for osteoporosis drug development.

ANIMAL MODELS

All laboratory animal models of human disease are imperfect. Finding differences between human and animal biology does not invalidate the animal model if these differences are understood and appropriate benchmark studies are performed to validate the model. For example, treating OVX rats and mice with estrogens, SERMs and bisphosphonates each produce expected changes in bone mass. Teriparatide has been extensively studied in animals and has anabolic bone effects in male and female rats and mice of all strains examined (whether intact or ovariectomized), rabbits, greyhound and beagle dogs, and both cynomolgus and rhesus ovariectomized monkeys. KO mice with disruptions in genes coding for estrogen receptors, RANKL, cathepsin K and sclerostin all show bone changes consistent with drug actions.

Following the failure of elemental fluoride treatment to reduce fractures despite increasing BMD in a clinical trial during the late 1980s, US FDA 1994 guidelines mandated examination of future osteoporosis drug candidates in a large animal exhibiting osteonal remodeling. Ovariectomized cynomolgus and rhesus macaques soon became the species of choice and have been successfully employed to examine multiple drugs [Jerome and Peterson, 2001; Brommage et al., 2001; Smith et al., 2009]. With greater similarity to humans compared to rodents, monkey studies have the downside that treatments typically last at least 16 months. These studies include all measurement made in advanced clinical trials, including bone turnover markers, DEXA BMD, skeletal architecture by CT and dynamic bone histomorphometric parameters for iliac crest biopsies. After necropsy multiple bones are subjected to breaking strength and histomorphometry examination.

Monkey studies provided data supporting a key belief in bone biology involving one stimulus responsible for activating osteonal bone remodeling. Trabecular width increased dramatically in two studies examining bones from monkeys treated with teriparatide or PTH [1–84] for 16 to 18 months. Detailed histological examinations showed examples of thick trabeculae being split into two by central osteonal tunneling [Jerome et al., 2001; Miller et al., 2008], supporting the long-held belief that low nutrient concentrations present within the interior of bone far from capillary perfusion activate osteonal remodeling to increase blood perfusion.

Although skeletal genes and biochemical pathways are conserved among mammals, differences in drug target protein sequences can prevent evaluation of specific drugs in some species. Odanacatib is

inactive in rodents as it does not inhibit rodent cathepsin K enzymatic activity and preclinical studies were successfully performed in rabbits [Pennypacker et al., 2011] and monkeys. The anti-RANKL antibody denosumab does not inhibit rodent RANKL but is efficacious in knockin mice expressing human RANKL [Kostenuik et al., 2009].

BEYOND OSTEOPOROSIS

Progress is being made in developing treatments for bone diseases other than osteoporosis. Selected examples include 1) improving BMP2 potency for fracture healing by creating an Activin A/BMP2 chimeric protein [Yoon et al., 2014]; 2) enzyme replacement therapy for hypophosphatasia resulting from mutations in the tissue-nonspecific alkaline phosphatase *ALPL* gene by creating a bone-targeted recombinant TNSALP [Whyte et al., 2012]; and FGF receptor antagonists to block the hypophosphatemic actions of excessive FGF23 [Wöhrle et al., 2013].

BEYOND CLASSICAL DRUG TARGETS

The great physicist Niels Bohr is claimed to have quipped that '*Prediction is very difficult, especially about the future*" as technologies keep advancing. While this review has focused on classic views about drug targets, some speculation about exciting developments is merited. One safe near-term prediction is that peptide drugs will increasingly consist of natural molecules having amino acid substitutions designed to optimize receptor activation and pharmacokinetic properties. One dramatic example is the triagonist designed to simultaneously activate glucagon, GIP and GLP-1 receptors [Finan et al., 2015]. For osteoporosis, the PTHrP analogue alaboparatide has several carboxy-terminal amino acid replacements to optimize activity and avoid the requirement for refrigerated storage.

Future technologies might include antisense approaches to silence RNA translation and block microRNAs, thereby greatly expanding the druggable genome. Mipomersen is an oligonucleotide inhibitor of apolipoprotein B-100 synthesis approved for treating homozygous familial hypercholesterolemia.

DEVELOPING NEWS

WNTs have been identified as physiological substrates for the secreted enzyme NOTUM. This lipase inactivates WNTs by removing the essential palmitoleic acid group required for binding to FRIZZLED receptors [Kakugawa et al., 2015; Zhang et al., 2015]. Lexicon Pharmaceuticals consistently observed elevated cortical bone thickness and strength in *Notum* KO mice, developed both neutralizing antibodies and orally active small molecules that inhibit NOTUM, and showed that treating mice and rats with these inhibitors stimulated modeling-dependent endocortical bone formation and thereby increased thickness and strength at multiple cortical bone sites (including the spine and femoral neck). Plans are underway to present Lexicon's data at the 2015 ASBMR conference.

ACKNOWLEDGMENTS

The author declares no conflicts of interest and is currently employed at the Institute of Experimental Genetics, German Mouse Clinic, Helmholtz Center Munich, 85764 Neuherberg, Germany. Thanks to former Lexicon colleagues, including Gwenn M. Hansen, Jeff Liu, Laura L. Kirkpatrick, Faika Mseeh, David Potter, David R. Powell, Melanie K. Shadoan, Andrea Y. Thompson, and Peter Vogel and Brian Zambrowicz and colleagues in the bone field, including Patrick Ammann, Cedo M. Bagi, Henry U. Bryant, David Ke, Christopher P. Jerome, Donald B. Kimmel, Michaela Kneissel, Scott C. Miller, Subbaramen Mohan, Susan Y. Smith, David D. Thompson, and Thomas J. Wronski for many educational discussions.

REFERENCES

Aggarwal S. 2013. Skeletal dysplasias with increased bone density: Evolution of molecular pathogenesis in the last century. Gene 528:41–45.

Augustine M, Horwitz MJ. 2013. Parathyroid hormone and parathyroid hormone-related protein analogs as therapies for osteoporosis. Curr Osteoporos Rep 11:400–406.

Ayturk UM, Jacobsen CM, Christodoulou DC, Gorham J, Seidman JG, Seidman CE, Robling AG, Warman ML. 2013. An RNA-seq protocol to identify mRNA expression changes in mouse diaphyseal bone: Applications in mice with bone property altering Lrp5 mutations. J Bone Miner Res 28:2081–2093.

Baron R, Kneissel M. 2013. WNT signaling in bone homeostasis and disease: From human mutations to treatments. Nat Med 19:179–192.

Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP. 2002. High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521.

Brommage R. 2001. Perspectives on using nonhuman primates to understand the etiology and treatment of postmenopausal osteoporosis. J Musculoskelet Neuronal Interact. 1:307–325.

Brommage R, Liu J, Hansen GM, Kirkpatrick LL, Potter DG, Sands AT, Zambrowicz B, Powell DR, Vogel P. 2014. High-throughput screening of mouse gene knockouts identifies established and novel skeletal phenotypes. Bone Research 2:14034.

Brunham LR, Hayden MR. 2013. Hunting human disease genes: Lessons from the past, challenges for the future. Hum Genet 132:603–617.

Chang MK, Kramer I, Huber T, Kinzel B, Guth-Gundel S, Leupin O, Kneissel M. 2014a. Disruption of Lrp4 function by genetic deletion or pharmacological blockade increases bone mass and serum sclerostin levels. Proc Natl Acad Sci USA 111:E5187–E5195.

Chang MK, Kramer I, Keller H, Gooi JH, Collett C, Jenkins D, Ettenberg SA, Cong F, Halleux C, Kneissel M. 2014b. Reversing LRP5-dependent osteoporosis and SOST deficiency-induced sclerosing bone disorders by altering WNT signaling activity. J Bone Miner Res 29:29–42.

Davisson MT, Bergstrom DE, Reinholdt LG, Donahue LR. 2012. Discovery genetics - The history and future of spontaneous mutation research. Curr Protoc Mouse Biol 2:103–118.

Farber CR, Clemens TL. 2013. Contemporary approaches for identifying rare bone disease causing genes. Bone Research 1:301–310.

Ferrari S. 2014. Future directions for new medical entities in osteoporosis. Best Pract Res Clin Endocrinol Metab 28:859–870.

Finan B, Yang B, Ottaway N, Smiley DL, Ma T, Clemmensen C, Chabenne J, Zhang L, Habegger KM, Fischer K, Campbell JE, Sandoval D, Seeley RJ, Bleicher K, Uhles S, Riboulet W, Funk J, Hertel C, Belli S, Sebokova E, Conde-Knape K, Konkar A, Drucker DJ, Gelfanov V, Pfluger PT, Müller TD, Perez-Tilve D, DiMarchi RD, Tschöp MH. 2015. A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. Nat Med 21:27–36.

Gregson CL, Steel SA, O'Rourke KP, Allan K, Ayuk J, Bhalla A, Clunie G, Crabtree N, Fogelman I, Goodby A, Langman CM, Linton S, Marriott E, McCloskey E, Moss KE, Palferman T, Panthakalam S, Poole KE, Stone MD,

Turton J, Wallis D, Warburton S, Wass J, Duncan EL, Brown MA, Davey-Smith G, Tobias JH. 2012. 'Sink or swim': An evaluation of the clinical characteristics of individuals with high bone mass. Osteoporos Int 23:643–654.

Hsu YH, Kiel DP. 2012. Clinical review: Genome-wide association studies of skeletal phenotypes: What we have learned and where we are headed. J Clin Endocrinol Metab 97:E1958–E1977.

Jerome CP, Burr DB, Van Bibber T, Hock JM, Brommage R. 2001. Treatment with human parathyroid hormone (1-34) for 18 months increases cancellous bone volume and improves trabecular architecture in ovariectomized cynomolgus monkeys (Macaca fascicularis). Bone 28:150–159.

Jerome CP, Peterson PE. 2001. Nonhuman primate models in skeletal research. Bone 29:1–6.

Joeng KS, Lee YC, Jiang MM, Bertin TK, Chen Y, Abraham AM, Ding H, Bi X, Ambrose CG, Lee BH. 2014. The swaying mouse as a model of osteogenesis imperfecta caused by WNT1 mutations. Hum Mol Genet 23:4035–4042.

Kakugawa S, Langton PF, Zebisch M, Howell SA, Chang TH, Liu Y, Feizi T, Bineva G, O'Reilly N, Snijders AP, Jones EY, Vincent JP. 2015. Notum deacylates Wnt proteins to suppress signalling activity. Nature 519:187–192.

Kang KS, Robling AG. 2015. New insights into Wnt-Lrp5/6-β-catenin signaling in mechanotransduction. Front Endocrinol (Lausanne) 5:246.

Kostenuik PJ, Nguyen HQ, McCabe J, Warmington KS, Kurahara C, Sun N, Chen C, Li L, Cattley RC, Van G, Scully S, Elliott R, Grisanti M, Morony S, Tan HL, Asuncion F, Li X, Ominsky MS, Stolina M, Dwyer D, Dougall WC, Hawkins N, Boyle WJ, Simonet WS, Sullivan JK. 2009. Denosumab, a fully human monoclonal antibody to RANKL, inhibits bone resorption and increases BMD in knock-in mice that express chimeric (murine/human) RANKL. J Bone Miner Res 24:182–195.

Leder BZ, O'Dea LS, Zanchetta JR, Kumar P, Banks K, McKay K, Lyttle CR, Hattersley G. 2015. Effects of abaloparatide, a human parathyroid hormone-related peptide analog, on bone mineral density in postmenopausal women with osteoporosis. J Clin Endocrinol Metab 100:697–706.

Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, Carew KS, Mane S, Najmabadi H, Wu D, Lifton RP. 2007. LRP6 mutation in a family with early coronary disease and metabolic risk factors. Science 315:1278–1282.

Maupin KA, Droscha CJ, Williams BO. 2013. A comprehensive overview of skeletal phenotypes associated with alterations in Wnt/β-catenin signaling in humans and mice. Bone Research 1:27–71.

McClung MR, Grauer A, Boonen S, Bolognese MA, Brown JP, Diez-Perez A, Langdahl BL, Reginster JY, Zanchetta JR, Wasserman SM, Katz L, Maddox J, Yang YC, Libanati C, Bone HG. 2014. Romosozumab in postmenopausal women with low bone mineral density. N Engl J Med 370:412–420.

Miller MA, Bare SP, Recker RR, Smith SY, Fox J. 2008. Intratrabecular tunneling increases trabecular number throughout the skeleton of ovariectomized rhesus monkeys treated with parathyroid hormone 1–84. Bone 42:1175–1783.

Movérare-Skrtic S, Henning P, Liu X, Nagano K, Saito H, Börjesson AE, Sjögren K, Windahl SH, Farman H, Kindlund B, Engdahl C, Koskela A, Zhang FP, Eriksson EE, Zaman F, Hammarstedt A, Isaksson H, Bally M, Kassem A, Lindholm C, Sandberg O, Aspenberg P, Sävendahl L, Feng JQ, Tuckermann J, Tuukkanen J, Poutanen M, Baron R, Lerner UH, Gori F, Ohlsson C. 2014. Osteoblast-derived WNT16 represses osteoclastogenesis and prevents cortical bone fragility fractures. Nat Med 20:1279–1288.

Niziolek PJ, Warman ML, Robling AG. 2012. Mechanotransduction in bone tissue: The A214V and G171V mutations in Lrp5 enhance load-induced osteogenesis in a surface-selective manner. Bone 51:459–465.

O'Brien CA, Plotkin LI, Galli C, Goellner JJ, Gortazar AR, Allen MR, Robling AG, Bouxsein M, Schipani E, Turner CH, Jilka RL, Weinstein RS, Manolagas SC, Bellido T. 2008. Control of bone mass and remodeling by PTH receptor signaling in osteocytes. PLoS ONE. 3(e):2942.

Ominsky MS, Niu QT, Li C, Li X, Ke HZ. 2014. Tissue-level mechanisms responsible for the increase in bone formation and bone volume by sclerostin antibody. J Bone Miner Res 29:1424–1430.

Overington JP, Al-Lazikani B, Hopkins AL. 2006. How many drug targets are there? Nat Rev Drug Discov 5:993–996.

Pennypacker BL, Duong LT, Cussick TE, Masarachia PJ, Gentile MA, Gauthier JY, Black WC, Scott BB, Samadfam R, Smith SY, Kimmel DB. 2011. Cathepsin K inhibitors prevent bone loss in estrogen-deficient rabbits. J Bone Miner Res 26:252–262.

Powell WF, Jr, Barry KJ, Tulum I, Kobayashi T, Harris SE, Bringhurst FR, Pajevic PD. 2011. Targeted ablation of the PTH/PTHrP receptor in osteocytes impairs bone structure and homeostatic calcemic responses. J Endocrinol 209:21–32.

Rhee Y, Allen MR, Condon K, Lezcano V, Ronda AC, Galli C, Olivos N, Passeri G, O'Brien CA, Bivi N, Plotkin LI, Bellido T. 2011. PTH receptor signaling in osteocytes governs periosteal bone formation and intracortical remodeling. J Bone Miner Res 26:1035–1046.

Saini V, Marengi DA, Barry KJ, Fulzele KS, Heiden E, Liu X, Dedic C, Maeda A, Lotinun S, Baron R, Pajevic PD. 2013. Parathyroid hormone (PTH)/PTH-related peptide type 1 receptor (PPR) signaling in osteocytes regulates anabolic and catabolic skeletal responses to PTH. J Biol Chem 288:20122–20134.

Sakharkar MK, Sakharkar KR. 2007. Targetability of human disease genes. Curr Drug Discov Technol 4:48–58.

Smith SY, Jolette J, Turner CH. 2009. Skeletal health: Primate model of postmenopausal osteoporosis. Am J Primatol 71:752–765.

Sobacchi C, Schulz A, Coxon FP, Villa A, Helfrich MH. 2013. Osteopetrosis: Genetics, treatment and new insights into osteoclast function. Nat Rev Endocrinol 9:522–536.

Spector R, Vesell ES. 2006. The heart of drug discovery and development: Rational target selection. Pharmacology 77:85–92.

St John HC, Meyer MB, Benkusky NA, Carlson AH, Prideaux M, Bonewald LF, Pike JW. 2015. The parathyroid hormone-regulated transcriptome in osteocytes: Parallel actions with 1,25-dihydroxyvitamin D3 to oppose gene expression changes during differentiation and to promote mature cell function. Bone 72:81–91.

Sule G, Campeau PM, Zhang VW, Nagamani SC, Dawson BC, Grover M, Bacino CA, Sutton VR, Brunetti-Pierri N, Lu JT, Lemire E, Gibbs RA, Cohn DH, Cui H, Wong LJ, Lee BH. 2013. Next-generation sequencing for disorders of low and high bone mineral density. Osteoporos Int 24:2253–2259.

Swinney DC. 2004. Biochemical mechanisms of drug action: What does it take for success?. Nat Rev Drug Discov 3:801–808.

Swinney DC. 2009. The role of binding kinetics in therapeutically useful drug action. Curr Opin Drug Discov Devel 12:31–39.

Tu X, Rhee Y, Condon KW, Bivi N, Allen MR, Dwyer D, Stolina M, Turner CH, Robling AG, Plotkin LI, Bellido T. 2012. Sost downregulation and local Wnt signaling are required for the osteogenic response to mechanical loading. Bone 50:209–217.

Urano T, Inoue S. 2014. Genetics of osteoporosis. Biochem Biophys Res Commun 452:287–293.

Van Wesenbeeck L, Van Hul W. 2005. Lessons from osteopetrotic mutations in animals: Impact on our current understanding of osteoclast biology. Crit Rev Eukaryot Gene Expr 15:133–162.

Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M, Mortier G, Mundlos S, Nishimura G, Rimoin DL, Robertson S, Savarirayan R, Sillence D, Spranger J, Unger S, Zabel B, Superti-Furga A. 2011. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A 155A:943–968.

Wergedal JE, Kesavan C, Brommage R, Das S, Mohan S. 2015. Role of WNT16 in the regulation of periosteal bone formation in female mice. Endocrinology 156:1023–1032.

Whyte MP, Greenberg CR, Salman NJ, Bober MB, McAlister WH, Wenkert D, Van Sickle BJ, Simmons JH, Edgar TS, Bauer ML, Hamdan MA, Bishop N, Lutz RE, McGinn M, Craig S, Moore JN, Taylor JW, Cleveland RH, Cranley WR, Lim R, Thacher TD, Mayhew JE, Downs M, Millán JL, Skrinar AM, Crine P,

Landy H. 2012. Enzyme-replacement therapy in life-threatening hypophosphatasia. N Engl J Med 366:904–913.

Wöhrle S, Henninger C, Bonny O, Thuery A, Beluch N, Hynes NE, Guagnano V, Sellers WR, Hofmann F, Kneissel M, Graus Porta D. 2013. Pharmacological inhibition of fibroblast growth factor (FGF) receptor signaling ameliorates FGF23-mediated hypophosphatemic rickets. J Bone Miner Res 28:899–911.

Yoon BH, Esquivies L, Ahn C, Gray PC, Ye SK, Kwiatkowski W, Choe S. 2014. An activin A/BMP2 chimera, AB204, displays bone-healing properties superior to those of BMP2. J Bone Miner Res 29:1950–1959.

Zhang X, Cheong SM, Amado NG, Reis AH, MacDonald BT, Ziebisch M, Jones EY, Abreu JG, He X. 2015. Notum is required for neural and head induction via Wnt deacylation, oxidation and inactivation. Dev Cell 32:719–730.

Zhao L, Shim JW, Dodge TR, Robling AG, Yokota H. 2013. Inactivation of Lrp5 in osteocytes reduces Young's modulus and responsiveness to the mechanical loading. Bone 54:35–43.

Zhou F, Meng S, Song H, Claret FX. 2013. Dickkopf-1 is a key regulator of myeloma bone disease: opportunities and challenges for therapeutic intervention. Blood Rev 27:261–267.