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REVIEW

Proteomics: an emerging tool for the discovery of bone mineral density molecular pathways

Fawzy Ali Saad[1]

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Abstract

Osteoporosis is a polygenic disease associated with low bone mineral density and deterioration of bone miniscule architecture and increased chance of bone fractures. However, several signaling pathways regulate bone mineral density including parathyroid hormone (PTH), Core-binding factor α-1 (CBFA1), Wnt/β-catenin, the receptor activator of the nuclear factor kappa-B (NF-κB) ligand (RANKL), myostatin, estrogen, and osteogenic exercise signaling pathways. These signaling pathways occur at protein level that depends not only on messenger RNA transcriptional regulation but also on a number of translational and posttranslational controls. Moreover, proteomic alterations in bone tissue due to a disease may occur in several ways that are unpredictable from either genome or transcriptome analysis. Decades of genome and transcriptome analyses have identified few causative genes; nonetheless, the majority of osteoporosis susceptibility genes remain unknown. It appears that a deeper view of bone proteome alterations will influence bone health and disease. This article highlights the efficacy of proteomics as an emerging tool for the discovery of bone mineral density molecular pathways.

Keywords Bone mineral density · Bone formation pathway · Bone resorption pathway · Osteoporosis · Bone metabolic diseases · Bone stem cells · Bone proteome

Introduction

The process of bone formation is highly regulated and involves the differentiation of mesenchymal stem cells (MSCs) into osteoblasts under the control of Core binding factor α1 (*CBFA1* or *RUNX2*) and Osterix (*OSX*) transcription factors. However, several other transcription factors are involved in osteoblast regulation including Hedgehog, Distal-less homeobox 5 (*Dlx5*), *TWIST1* (a basic helix-lop-helix transcription factor), activating transcription factor 4 (*ATF4*), special AT-rich sequence-binding protein 2 (SATB2), and Schnurri-3 (*SHN3*) (St-Jacques et al. 1999; Acampora et al. 1999; Bialek et al. 2004; Yang et al. 2004; Dobreva et al. 2006; Jones et al. 2006). In addition, mesenchymal stem cells differentiate through specific signaling pathways into chondrocytes, and adipocytes (Ashton et al. 1980; Friedenstein et al. 1982; Madras et al. 2002; Bianco and Robey 2015). Osteoblasts growth and maturation are regulated temporally and spatially by different transcription factors (Stein et al. 1996; Saad 2012). However, little is yet known about the mysterious process of bone matrix mineralization; the transport and deposit of precise ratios of inorganic minerals within the organic extracellular matrix (Blair et al. 2007; Tsai and Chan 2011). Therefore, understanding the molecular events leading to bone matrix mineralization is clinically relevant to metabolic bone diseases, tissue bioengineering, and gene therapy. There are several excellent reviews covering osteoporosis prevalence and epidemiology, BMD and bone loss; bone homeostasis; bone remodeling, and the different regulatory factors and pathways (Saad 2012; Saad 2013; Leslie and Morin 2014; Saad 2020; Al-Bari and Al-Mamun 2020; Zhang et al. 2020). While gene therapy applications for bone regeneration are in early stages, pioneer studies have established that genetically modified muscle and fat grafts are capable to repair large defects in bone (Evans et al. 2009).

Peak bone mass is the maximum deposit of inorganic minerals within bone organic extracellular matrix during bone development and growth, which normally occurs during the third decade of life (Chew and Clarke 2018). Regular physical activity during childhood stimulates peak bone

✉ Fawzy Ali Saad
fa_saad@yahoo.co.uk

[1] Department of Orthopaedic Surgery, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA

mass to reach its maximum potential; particularly at the time of puberty where the skeleton is relatively sensitive to mechanical signals stimulated by osteogenic exercise (Hingorjo et al. 2008). Lifestyle factors such as physical activity, sedentary way of life, dietary calcium intake, consumption of calcium depleting drinks (Alcohols, acidic drinks, caffeinated beverages, or carbonated water), acidic foods, and smoking among others influence 20–40% of adult peak bone mass (McGartland et al. 2003; Ma and Jones 2004; Kristensen et al. 2005; Libuda et al. 2008; Weaver et al. 2016).

While about 39% of total body bone mineral deposits are achieved during the 4 years around peak bone mass, 95% of adult bone mass is acquired by the 4th year after peak bone mass (Baxter-Jones et al. 2011). Blood calcium deficit is a detrimental factor to bone mass as it stimulates the parathyroid cells to release Parathyroid hormone, which induces bone resorption and calcium release into blood streams to equilibrate blood calcium concentration to a physiologic range of 88–104 mg per liter (mg/L); 2.2–2.6 mM (Peacock 2010; Yu and Sharma 2020). Parathyroid hormone promotes bone resorption through inducing the receptor activator of NF-κB (RANK) ligand (RANKL), while inhibiting osteoprotegerin, the decoy receptor of RANKL. Conversely, blood calcium upsurge promotes thyroid gland C cells to release Calcitonin; a 32 amino acid hormone, which stimulates calcium deposition and bone formation, while inhibits osteoclast activity and bone resorption.

Bone mass changes with age, having a rapid increase during the childhood to reach a peak level by mid or late twenties in life and declines later in women and elder men. Bone loss due to loss of inorganic minerals and organic extracellular matrix starts around the age of 40 in both genders at less than 1% a year of bone mass. In women, unfortunately bone loss increases rapidly after menopause as Estrogen cessation unleashes bone resorption (Riggs 2000), consequently, the rate of trabecular bone loss can surge up to 6% a year, with a greater loss in the first 5 years of postmenopause (Hingorjo et al. 2008).

Bone formation and resorption signaling pathways regulate bone remodeling. Bone loss is caused by unbalanced bone remodeling where bone resorption surpasses bone formation due to ageing and decline in sex hormones (Riggs et al. 1969; Riggs 2000), which increases the risk for osteoporosis and bone fracture. Proinflammatory cytokines are responsible for the induction of both inflammatory bowel disease and bone loss associated with the disease. Levels of the proinflammatory cytokines Nuclear factor-kappa-B (NF-KB), Tumor necrosis factor alpha (TNF-α), Interleukin 1 beta (IL1β), IL6 and IL17 are increased in the serum of inflammatory bowel disease patients (Mahida et al. 1989; Ardite et al. 1998; Paganelli et al. 2007; De Vry et al. 2007; Ozaki et al. 2012), which dictates the use of anti-inflammatory agents. Inflammation is the key factor of determining low bone mineral density in pediatric inflammatory bowel disease (Paganelli et al. 2007). Therefore, children and adolescents with inflammatory bowel disease may not reach a potential bone mass peak, which puts them at greater risk for osteoporotic fractures. Also, there is a high rate of bone resorption in patients with multiple myeloma due to the activation of RANK (Receptor activator of nuclear factor-kappaB) and RANK Ligand (Roux and Mariette 2004).

Bone remodeling is the collective activity of osteoclasts and osteoblasts; the bone resorbing and forming cells respectively (Hinoi et al. 2006). Unbalanced bone remodeling is a key factor in determining bone strength and weakness, and leads to metabolic bone disorders with either high or low bone mass such as osteopetrosis or osteoporosis (Manolagas and Jilka 1995). Nevertheless, the molecular mechanisms underlying bone remodeling remain poorly understood.

While primary osteoporosis is due to ageing and subsequent decline in sex hormones (estrogen, progesterone, androgens and testosterone), secondary osteoporosis emerges either as an outcome of other diseases or as a side effect of prescription medications (Mirza and Canalis 2015). Osteoporosis is a painless disease, which develops invisible through years of bone loss leading to weak and fragile bone (Abdulameer et al. 2012). Therefore, the silent killer progresses without symptoms until a fracture occurs (Parsons 2005; Szamatowicz 2016; Al Anouti et al. 2019; Saad 2020). Osteoporosis is a polygenic disorder influenced by multiple genes and environmental risk factors, each with a modest effect on bone mass and susceptibility to fracture. The complex architecture of osteoporosis molecular genetics is a challenging topic to explore; however, novel insights into this complex architecture have been recently emphasized (Saad 2020).

Osteoporosis is an osteodegenerative disease associated with low bone mineral density (BMD) and deterioration of bone minute architecture with increased chance of fracture (Albagha and Ralston 2006; Ralston and Uitterlinden 2010). Worldwide, there are approximately 200 million people affected with osteoporosis (Reginster and Burlet 2006; Al Anouti et al. 2019). Additionally, the number of diabetics exceeds 422 million and there are 46.8 million affected with Alzheimer disease. These individuals are prone to bone fractures (Melton et al. 1994; Kanna and Roffe 2006; Sealand et al. 2013; Cornelius et al. 2014; Rubin 2017), which suggest a common link between osteoporosis and these diseases (Woodman 2013; Khan and Fraser 2015). In the United States, osteoporosis causes more than 2 million fractures annually with estimated annual expenditures of $19 billion. Moreover, the fracture burden and its related costs are expected to duplicate by 2025 (Burge et al., 2007; Becker et al. 2010). In the European Union, osteoporosis causes more than 3.5 million fractures every year, with an annual estimated cost of €37 billion (Hernlund et al. 2013).

Unfortunately, due to the rapid growth of the globe ageing population, the socioeconomic cost of osteoporotic fractures would increase worldwide. Moreover, osteoporotic fractures cause an annual global loss of 5.8 million healthy individuals to disability (IOF report 2014).

So far, there is no single safe medication for osteoporosis in the drug market (Saad 2020). In fact, current osteoporosis prescription medications have serious adverse events; some of which represent a real danger to life (Hough et al. 2014), which illustrate the urgent need for safe drugs. Therefore, advances in the knowledge about the molecular pathways regulating bone mineral density are essential for understanding the pathogenesis of osteoporosis and may provide the means to develop anabolic therapies for osteoporosis (Saad 2012). This article highlights the efficacy of proteomics as an emerging technology for the discovery of bone mineral density pathways.

The discovery of bone mineral density pathways

Genome and transcriptome analyses are common tools for the discovery of genes influencing bone mineral density. Recently, proteome analysis has emerged as a new tool for the discovery of genes underlining genetic diseases (Sellers and Yates 2003). The advantages and limitations of these tools are detailed elsewhere (Saad 2013). The density of bone minerals in the specific area reflects bone mineral density (BMD). Although BMD is considered the surrogate phenotype for the risk of osteoporosis and bone fracture, a great deal of fracture risk is independent of BMD (Marshall et al. 1996; Duan et al. 2006; Seeman 2007). BMD is a multifactorial phenotype. It depends on genetic and environmental risk factors, and their interaction with each other. These factors shall determine skeletal health throughout the life.

The genetic components of osteoporosis represent about 50–80%, which depend on distinct anatomical location (Sigurdsson et al. 2008; Ralston and Uitterlinden 2010). The environmental risk factors include calcium deficient diet, decline of sex hormones, and sedentary lifestyle (Koromani et al. 2019; Herbert et al. 2019) among others.

Bone tissue proteomics

The proteome of neurodegenerative diseases are available (Ping et al. 2018). Therefore, it is anticipated that the proteome of bone diseases will become available in the near future. Bone mineral density depends on the balance between CBFA1 bone formation and RANKL bone resorption signaling pathways. While successful application of antibody microarray to analyze protein expression of the squamous cell carcinomas of the oral cavity has been reported (Knezevic et al. 2001), bone tissue proteomics is still lagging behind soft tissues and biofluids proteomics. This lagging is due to bone intricate structure and biochemistry. Proteomics analysis holds a great advantage over genomic and transcriptomic analyses. Signaling pathways occur at protein level that are not predictable through genomic or transcriptomic analysis. Furthermore, the correlation between genome or transcriptome and proteome is insignificant or inexistent, which make proteomic analysis more appropriate for pathways discovery. The molecular pathways regulation bone mineral density and their complex interplay with each other are illustrated in Fig. 1.

CBFA1 bone formation pathway

The osteoblast master transcription factor, *CBFA1*, regulates osteoclast and osteoblast functions during bone remodeling. *CBFA1* regulates *Osterix* transcription through direct binding to its promoter.

Bone stem cells and bone iPSCs proteomics Two-dimensional gel electrophoresis and mass spectrometric analysis of human adipose stem cells (ASC) induced for differentiation into osteoblasts; induced pluripotent stem cells (iPSCs), have identified 51 differentially expressed proteins under distinct experimental conditions. Sixteen silver stained spots were identified in the absence of stimulation, while 28 silver stained spots were identified after 4 weeks of osteogenic stimulation. Similarly, seven silver stained spots were identified after 2 weeks of osteogenic stimulation compared with no stimulation or 4 weeks of osteogenic stimulation (Giusta et al. 2010).

Two-dimensional gel electrophoresis and mass spectrometric analysis revealed 52 proteins responsible for the differentiation of mesenchymal stem cells into osteoblasts (Zhang et al. 2007). These proteins fit into several groups including metabolism, transcription, protein folding, calcium-binding proteins, protein decay, and signal transduction pathways.

Label free mass spectrometry and quantitative proteomic analysis of how proinflammatory cytokines modulate mesenchymal stem cells secretome revealed that proinflammatory cytokines have a strong impact on human bone marrow-derived MSC secretome; however, the majority of the induced cytokines are involved in inflammation, angiogenesis, or both. Moreover, further functional analysis revealed a role of Metalloproteinase 1 (MP1) in the antiangiogenic property of inflammatory stimulated MSC (Maffioli et al. 2017).

Flow cytometric analysis of mesenchymal stem cells derived from human bone marrow aspirate and peripheral blood monocytes of the same patient revealed that the proportion of MSC ($CD34^{-}/CD29^{+}/CD105^{+}$) and osteogenic

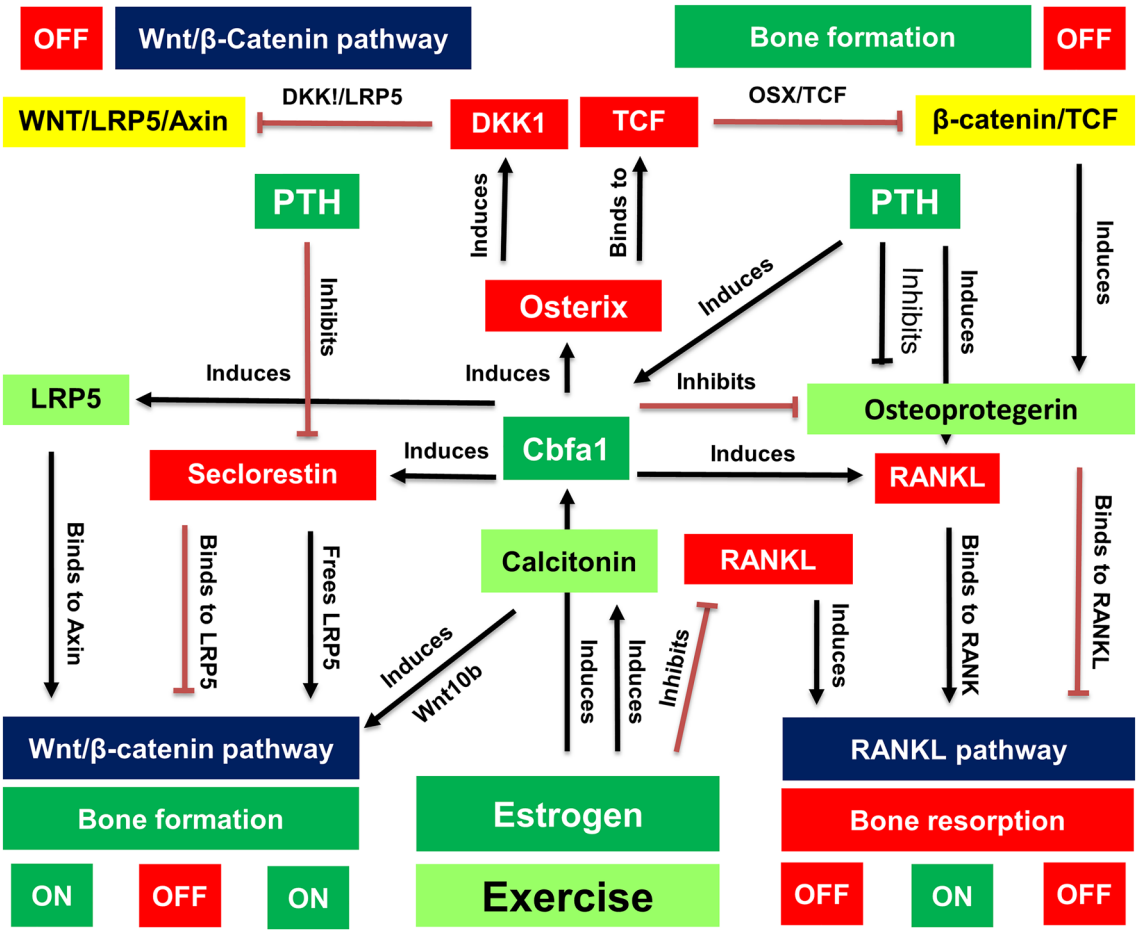

Fig. 1 The signaling pathways regulating bone mineral density. PTH inhibits Sclerostin while inducing Cbfa1 expression, which simultaneously activates the Wnt/β-catenin and Cbfa1 bone formation pathways. PTH increases the expression of RANKL and bone resorption. Cbfa1 promotes osteoclast differentiation by inducing RANKL and inhibiting OPG. Cbfa1 directs the expression of LRP5 and Sclerostin, which, respectively, activate and inhibit the Wnt/β-catenin signaling pathway. β-Catenin interacts with LCF/TCF proteins to induce OPG expression in osteoblasts, which consequentially inhibits bone resorption. Cbfa1 induces Osterix expression through direct binding to its promoter. Osterix activation induces DKK1 expression via direct binding to its promoter, which leads DKK1 to bind LRP5 and inactivate the Wnt/β-catenin signaling pathway. In addition, Osterix directly binds and disrupts TCF ability to bind DNA, which blocks the binding between TCF and β-catenin to transactivate the Wnt/β-catenin target genes. Osteogenic exercise inhibits bone resorption and induces bone formation pathways by inhibiting myostatin and inducing irisin and estrogen. Myostatin inhibition halts the bone resorption pathway, while Irisin induction stimulates the Cbfa1 and β-catenin bone formation pathways. Estrogen inhibits RANKL and bone resorption, while induces Calcitonin and Cbfa1 that promotes calcium deposit and bone formation. Reprinted with modification from Saad (2020) (https://doi.org/10.1111/nyas.14327) under License Number 5086000273276 from John Wiley and Sons

factors were higher in bone marrow aspirate than peripheral blood monocytes. Mass spectrometry and Western blot analysis indicated that the levels of the osteoclast inhibitor catalase and the osteogenic marker Glutathione peroxidase 3 (GPX3) were higher in bone marrow aspirate than peripheral blood monocytes (Niu et al. 2014).

Two-dimensional gel electrophoresis of bone marrow mesenchymal stem cells stimulated or unstimulated with Bone morphogenetic protein 2 (BMP2) revealed 20 silver stained spots. Mass spectrometric analysis identified 9 downregulated and 11 upregulated proteins after stimulation with recombinant human BMP2 (rhBMP2). The upregulation of Lim and SH3 domain protein 1 (LASP1) and the downregulation of ferritin (FRTN) were verified by Western blot and real-time RT-PCR (Hu et al. 2014).

Chondrocyte proteomics Chondrocytes are responsible for long bone formation. Two-dimensional gel electrophoresis of human chondrocytes stimulated with Interleukin 1β (IL1β) and/or Tumor necrosis factor α (TNFα) revealed 37 silver stained spots. Further analysis by mass spectrometry (MS) or MS/MS identified 35 different proteins. While IL1β modulates 22 proteins, TNFα modulates 20 proteins, as compared with unstimulated chondrocytes. In addition, 18

proteins were modulated by both IL1β and TNFα (Cillero-Pastor et al. 2010).

Two-dimensional gel electrophoresis combined with mass spectrometric analysis of the hyaluronic acid protective effects on osteoarthritis chondrocytes have identified 13 silver stained spots corresponding to 12 Hyaluronic acid (HA) regulated proteins in osteoarthritis chondrocytes under oxidative stress. The differential expression of the hyaluronic acid regulated proteins transaldolase (TALDO), annexin A1 (ANXA1), and Elongation factor 2 (EF2) was verified by Western blot of the control and HA treated osteoarthritis chondrocytes (Yu et al. 2014).

Isobaric tags for relative and absolute quantitative proteomic analyses of the effects of antler extracts on primary chondrocyte biology revealed significant increase of the proliferation markers Ki-67 (MKI67) and Stathmin1 (STMN1), differentiation inhibitor Tartrate-resistant acid phosphatase (TRAP/ACP5), and the apoptosis inhibitors NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4-like 2 (NDUFA4L2), and reticulocalbin 1 (RCN1) (Yao et al. 2019).

Osteoblast proteomics Osteoblasts are responsible for the synthesis of bone extracellular matrix, bone matrix mineralization, and bone formation. Analysis of MC3T3-E1 mouse osteoblast cells after inorganic phosphate treatment by cleavable isotope-coded affinity tag (ciCAT) reagents, strong cation-exchange (SCX) liquid chromatography (SCX-LC), and mass spectrometry identified 7227 unique peptides corresponding to 2501 proteins, which roughly represent 9% of the mouse genome encoded proteins (Conrads et al. 2004).

Proteomic analysis of differentiating mouse MC3T3-E1 osteoblast cells identified several proteins which play roles in the cytoskeleton scaffold assembly. IQ domain GTPase-activating protein 1 (IQGAP1), gelsolin, moesin, radixin, and cofilin-1 were among the upregulated proteins. Similarly, focal adhesion signaling pathway analysis revealed that filamin A (FLNA), laminin alpha 1 (LAMA1), LAMA5, Collagen type I alpha 1 (COL1A1), COL3A1, COL4A6, and COL5A2 were upregulated; whereas COL4A1, COL4A2, and COL4A4 were downregulated (Hong et al. 2010).

Proteomic differential display and mass spectrometric analysis identified a number of differentially expressed proteins in mineralizing 7F2 mouse osteoblast cells (Saad and Hofstaetter 2011). One of these proteins was among the proteins responsible for the differentiation of mesenchymal stem cells into osteoblasts (Zhang et al. 2007). Similarly, three of these proteins (vimentin, calreticulin, and Lamin a/c) have known biological functions in osteoblast differentiation (Shapiro et al. 1995; Szabo et al., 2008; Akter et al. 2009), which further confirm their roles in bone formation.

Exosomes are cellular nanostructure vesicles originate mostly from the plasma membrane, which are released by most cell types and play roles in intercellular communications and biotic cargo transfers. Proteomic analysis of mouse MC3T3-E1 osteoblast cells exosomes has identified 1069 proteins of which 786 overlap with ExoCarta database. The eukaryotic initiation factor 2 (EIF2); an important player in bone formation, was among these proteins. Gene ontological analysis revealed that these exosomes are mainly involved in intracellular signaling and protein subcellular localization (Ge et al. 2015).

Cellular transdifferentiation through genetic reprogramming offers new opportunities in the field of cell replacement therapy and tissue bioengineering. Myoblasts transdifferentiate into osteoblasts upon BMP2 stimulation through the activation of CBFA1 bone formation pathway. Proteomic analysis of mouse C2C12 premyoblast cells after BMP2 stimulation (iPSCs) has identified 1321 potential phosphoproteins in stage one (stimulation for 30 min), and 433 proteins were quantified in stage two (stimulation for 3 days). Among these proteins, 374 BMP2-specific phosphoproteins and 54 differentially expressed proteins (Kim et al. 2009).

Bone proteomics Exploring bone proteome is vital for revealing the mechanisms regulating bone homeostasis in health and disease. Mass spectrometry profiling of rat bone extracellular matrix proteins revealed the presence of 133 proteins (108 in the metaphysis and 25 proteins in the diaphysis). Twenty-one of these 133 proteins are bone specific including osteopontin, bone sialoprotein, osteocalcin, osteoregulin, Collagen type I, and Collagen type II (Schreiweis et al. 2007). Attractively, Collagen type II, a cartilage-specific protein, was identified in metaphysis and diaphysis. This attractive observation was validated by Western blot. Proteomic analysis of osteonecrotic femoral head revealed 197 proteins. Of these proteins, 141 are upregulated and 56 are downregulated (Zhang et al. 2009).

High-sensitive, high-resolution tandem mass spectrometry was performed on ancient proteins extracted from a 430 century old woolly mammoth bone preserved in the Siberian permafrost. This sophisticated mass spectrometric analysis identified 126 unique low-abundance extracellular matrix and plasma proteins (Cappellini et al. 2012).

Some drugs like glucocorticoids impair osteoblast differentiation and bone formation leading to low bone mineral density and induction of secondary osteoporosis. Proteomic analysis of MC3T3-E1 mouse osteoblast cells treated with the glucocorticoid dexamethasone revealed an increase in the expression of Tubulins (TUBA1A, TUBB2B, and TUBB5), S100 proteins (S100A11, S100A6, S100A4, and S100A10), Myosins (MYH9 and MYH11), IQGAP1, and apoptosis and stress proteins. Proteomic analysis further revealed a decrease in the expression of ATP synthases (ATP5O, ATP5H, ATP5A1, and ATP5F1), Ras-GTPase activating protein SH3 domain binding protein 1 (Ras-G3BP1),

and Ras-related proteins (RAB-1A, RAB-2A, and RAB-7). Such proteomic profile may be collectively responsible for glucocorticoid-induced osteoporosis (Hong et al. 2011).

RANKL bone resorption pathway

The receptor activator of NF-κB (RANK), its ligand (RANKL), and Osteoprotegerin (OPG); the decoy receptor of RANKL, regulate bone resorption signaling pathway. While binding between RANKL and RANK promotes RANKL bone resorption pathway, binding of OPG to RANKL blocks the ability of RANKL to bind its receptor RANK, which prevents osteoclastogenesis and RANKL bone resorption pathway. A comprehensive reaction map of RANKL signaling pathway is available, which might provide novel insights into bone disease pathophysiology and may lead to the discovery of new biomarkers (Raju et al. 2011).

Osteoclast proteomics Osteoclasts are the cells responsible for degrading and resorbing bone. Lipid rafts play a crucial role in cell fusion upon RANKL induction of osteoclast differentiation and maturation to multinucleated bone resorbing cells. Two-dimensional gel electrophoresis and MALDI-TOF mass spectrometry of lipid rafts have identified 12 functional proteins among 34 silver stained spots. Of these 12 proteins, a subunit of Vacuolar H(+)-ATPase (V-ATPase) was identified at an approximate molecular weight of 56.94 kDa and pI of 5.4. V-ATPase has been recognized for its role in bone resorption pathway (Ryu et al. 2010).

Osteoclasts secrete acid hydrolases into the bone resorption lacuna where bone degradation occurs. Proteomic analysis of acid hydrolases secreted by osteoclasts during the induction of mouse myeloid Raw 264.7 cell line with RANKL revealed an increase of mannose 6-phosphate-containing acid hydrolases secretion after the differentiation of Raw 264.7 cells into mature osteoclasts. Secreted proteins were run into a mannose 6-phosphate receptor affinity column. Proteomic analysis of the captured proteins revealed 58 different acid hydrolases, 16 of which are involved in bone homeostasis; however, the expression of other 42 remained stable during osteoclastogenesis (Czupalla et al. 2006).

Proteomic analysis of myeloid Raw 264.7 mouse cells differentiation into osteoclast-like in response to RANKL induction has identified more than 4000 proteins. Among these, 138 were novel osteoclast-related proteins. Further proteomic analysis revealed that cystathionine γ-lyase (Cth/CSE), epidermal growth factor (EGF)-like repeat, discoidin I-like domain-containing protein 3, Integrin α phenylalanyl-glycyl–glycyl-alanyl-prolyl (FG-GAP) repeat containing 3, adseverin, and serpin b6b (Serpinb6b) expression were increased during osteoclastogenesis (Itou et al. 2014).

Bone mineral density biomarkers Sequential protein extraction followed by automated 2D-LC–MS/MS analysis has identified 6202 unique peptides, which belong to 2479 unique proteins. Among these unique proteins, over 40 bone-specific proteins and 15 potential biomarkers were already known. These biomarkers include osteocalcin, cathepsins (A, D, G and K), matrix metalloproteinases (MMP 2 and 19), and plasminogen (Jiang et al. 2007). Proteomic analysis of Mexican postmenopausal women serum reveals vitamin D-binding protein (VDBP) as a potential biomarker for low bone mineral density (Martínez-Aguilar et al. 2019). High osteocalcin levels are associated with high bone mineral density. All the other markers including low osteocalcin levels are associated with low mineral density.

Discussion

While numerous genes causing skeletal disorders have been identified through the study of rare monogenic diseases (Bonafe et al. 2015); a small number of osteoporosis vulnerability genes have been identified through this procedure (Costantini and Mäkitie 2016).

Moreover, identification of the genes influencing low bone mineral density by genomic means proved difficult with limited success (Farber 2012). Indeed, decades of genome and transcriptome analyses have identified few causative genes; however, the majority of osteoporosis vulnerability genes remain unknown (Costantini and Mäkitie 2016). Therefore, decades after revealing the human genome sequence, the great promise to excavate the complex architecture of osteoporosis molecular genetics through genomic means has been difficult to achieve.

The correlation between genomic DNA or mRNA and protein levels in a cell is either insignificant (Huang et al. 2003; Sellers and Yates 2003; Maier et al. 2009) or upright does not exist (Yeung 2011), which is due to transcriptional regulation, alternative splicing, translational controls, post-translational modifications, and protein decay. Since genes influence disease through the proteins they encode, proteomics represents a powerful tool to discover genes underlining a genetic disease (Sellers and Yates 2003). In addition, the expression levels of all proteins in a cell provide the most relevant data set characterizing a biological system (Cox and Mann 2007). Therefore, the use of genome and transcriptome analyses for bone mineral density pathways discovery may face several challenges.

While calls for proteomic profiling of human diseases have been made about two decades ago (Hanash 2003; Sellers and Yates 2003), applying proteomics for bone mineral density pathways discovery is in early stages. Applying proteomics to investigate bone diseases offers the prospect that proteomics will overcome the limitations of genome and

transcriptome analyses (Petricoin et al. 2002). Therefore, unlike genome and transcriptome analyses, proteomics offers the opportunity to fulfil the unfilled promise to excavate the complex architecture of bone mineral density and osteoporosis. Similarly, RNA interference, CRISPR interference, CRISPR Cas9, or gene targeting technology is a powerful tool for the rapid analysis of protein functions in cellular or animal models (LePage and Conlon 2006; Seibler and Schwenk 2010).

Conclusions

The dynamic properties of bone tissue proteome provide an incentive to analyze gene expression of a bone disease at protein, rather than messenger RNA level. The application of proteomics in bone research holds a great promise to accelerate osteoporosis genes discovery and increase our understanding of protein expression, dynamics, decay, posttranslational modifications, and signal transduction pathways regulating bone mineral density. Identification of novel proteins that may be associated with bone matrix mineralization provides vital knowledge toward deciphering the mystery of this process. Moreover, proteomic profiling of mesenchymal stem cells, osteoblasts, chondrocytes, and osteoclasts is enriching our knowledge about the molecular pathways regulating bone growth and remodelling. Similarly, proteomics provides an efficient tool to explore the molecular mechanisms regulating bone mineral density. Recently, proteomic analysis has emerged as a powerful tool to identify protein involved in bone mineral density. Hopefully this initiative will help integrating proteomics in the study of bone diseases, which shall accelerate the discovery of gene associated with these diseases. Strategies to combine proteomics with RNA interference, CRISPR interference, or CRISPR Case9 gene inactivating technology would greatly improve the efficiency of gene discovery, rapidly elucidate gene functions, and identify pathways involved in the pathogenesis of bone diseases. Similar to the availability of Alzheimer's and Parkinson's disease proteome, it is anticipated that the proteome for bone diseases will be available in the near future.

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Conflict of interest The author declares no conflict of interest.

Ethics approval There were no patients enrolled for this study.

Consent to participate There were no patients enrolled for this study.

Consent for publication This is a single author manuscript.

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