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Mini-Review

Exploration for the molecular pathways that regulate bone matrix mineralization in the proteome era

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ABSTRACT

The complex process of bone matrix mineralization is tightly regulated by several transcription factors and signal transduction pathways. However, signal transduction pathways occur at protein level that depends not only on mRNA transcriptional regulation but also on a multitude of translational and posttranslational controls. Furthermore, proteomics allows a holistic view of complex molecular pathways and provides an efficient method to determine protein candidates and elucidate the signaling transduction pathways that regulate bone matrix mineralization.

KEYWORDS: mesenchymal stem cells, osteoblast matrix mineralization, bone mineral density, bone gene therapy, osteoporosis

ABBREVIATIONS

Dlx5, distal-less homeobox 5; ATF4, activating transcription factor 4; SATB2, special AT-rich sequence-binding protein 2; Twist1, a basic helix-loop-helix transcription factor

INTRODUCTION

Mesenchymal stem cells differentiate through specific signal transduction pathways into osteoblasts, chondrocytes and adipocytes [1-3]. Also, bone marrow-derived mesodermal progenitor cells differentiate into osteoblasts, chondrocytes, adipocytes, myocytes, and endothelial cells [4]. Osteoblasts are the bone forming cells that are responsible for synthesis, deposition, and mineralization of the extracellular matrix of bone. Bone forms through endochondral ossification that involves a cartilage anlagen and intra-membranous ossification that forms directly from mesenchymal cells condensations. The process of bone formation is highly regulated and involves the differentiation of mesenchymal stem cells into osteoblasts under the control of Core binding factor α-1 (Cbfa1) and Osterix (Osx) transcription factors. Osteoblast commitment and differentiation are regulated by several transcription factors and complex signal transduction pathways that elicit a cascade of highly orchestrated gene expression. Osteoblast differentiation is essential for bone formation and defects in this process result in weak bone with an increased chance for fractures [5]. Osteoblast matrix mineralization is an important determinant of the stiffness and hardness of bone tissue [6]. Transcriptional control of osteoblast growth and differentiation is tightly regulated both temporally and spatially [7]. However, little is known about the molecular mechanisms that regulate bone matrix mineralization.

Understanding the molecular events leading to bone matrix mineralization is clinically relevant to bone metabolic diseases, tissue engineering and gene therapy. While gene therapy applications for bone regeneration are in early stages, pioneer studies by our group have established that genetically modified muscle and fat grafts are capable to repair defects in bone and cartilage [8].

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Bone remodeling is regulated by osteoclasts and osteoblasts; the bone resorbing and forming cells respectively [9]. The molecular mechanisms underlying this process are poorly understood. Unbalanced bone remodeling is a key factor in determining bone strength and weakness and leads to bone metabolic disorders such as osteopetrosis and osteoporosis [10]. Advances in the knowledge about the molecular mechanisms that control bone matrix mineralization are essential for understanding the pathogenesis of osteoporosis and may provide the means to develop anabolic therapies for bone metabolic diseases. Therefore, this minireview evaluates methods for exploring the complex pathways of bone matrix mineralization.

METHODS

Different approaches such as linkage analysis, gene microarray and proteomic analysis have been currently employed to identify genes involved in the complex pathways of bone matrix mineralization.

a) Genome analysis

Linkage analysis [reviewed in 11-13] has been very successful in identifying numerous quantitative trait loci involved in bone mineral density regulation but most of the genetic variables remain to be discovered, which illustrates the need for new strategies [14]. Moreover, linkage analysis has mainly failed to identify the causative genes of a complex genetic disorder such as osteoporosis [15].

b) Transcriptome analysis

Gene microarray has offered some insight into the global patterns of gene expression during osteoblast matrix mineralization *in vitro*, as well as osteoblast and osteoclast regulation, which is essential to comprehend the pathogenesis of bone metabolic diseases [16]. Furthermore, it provides a panoramic analysis of gene alterations underlying the complex process of bone formation [17]. Therefore, several groups have used microarray to unravel the molecular pathways that regulate osteoblast differentiation and bone formation using a number of cellular models [4, 18-33].

Moreover, Cbfa1 and Osx have been identified as master regulators of osteoblast differentiation and absence of either one, leads to complete lack of bone matrix mineralization [34-36]. Several other transcription factors are involved in osteoblast regulation including Hedgehog, Dlx5, Twist1, ATF4, SATB2 and Shn3 [37-42]. Also, other factors [43, 44], and signaling pathways [45] are essential for osteoblast differentiation. These regulators interact with each other to trigger diverse signals and orchestrate the transcription of genes crucial to define osteoblastic lineage and differentiation.

c) Proteome analysis

While the application of proteomics to bone field is in early stages, it promises to increase our understanding about the molecular pathways underlying the complex process of bone formation and remodeling. Mass spectrometric profiling of proteins present in the extracellular matrix of rat bone revealed the presence of 108 and 25 proteins in the metaphysis and diaphysis, respectively. Twenty-one of them were bone specific and appeared in both samples including: osteopontin, bone sialoprotein, osteocalcin, osteoregulin, and type I collagen [46].

Two dimension gel electrophoresis and mass spectrometric analysis revealed 52 proteins responsible for the differentiation of mesenchymal stem cells into osteoblasts [47]. These proteins were separated into several groups including metabolism, transcription, protein folding, calcium-binding proteins, protein degradation and signal transduction. Proteomic analysis of osteonecrotic femoral head bone tissues revealed 141 upregulated and 56 downregulated proteins compared to the controls [48].

Proteomic differential display and mass spectrometric analysis identified 16 differentially expressed proteins in mineralizing osteoblast [49]. One of these proteins, transketolase, was among the proteins responsible for the differentiation of mesenchymal stem cells into osteoblasts [47]. Additionally, vimentin, calreticulin and lamin a/c have been noted for biological functions in osteoblast differentiation [50-52], which further confirm their roles in osteogenesis.

CONCLUSIONS

Osteoporosis is a complex genetic disorder associated with low bone mineral density and deterioration of bone microarchitecture. Increased osteoclastogenesis and reduced osteoblastogenesis lead to low bone mineral density due to disparity between bone resorption and formation.

Linkage analysis has shown great success in identifying gene mutations causing monogenic bone diseases, and in identifying numerous quantitative trait loci involved in bone mineral density regulation. However, until now, few causative genes have been discovered and most of the genetic variables leading to osteoporosis remain to be discovered.

Gene microarray provides more information about mRNA transcriptional levels and explores the relationship between certain genes and the biological pathways that regulate bone matrix mineralization. The downsides of gene microarray are that signal transduction pathways occur at protein level and the abundance of mRNA is not a real indicator of a gene role in cellular functions.

The application of proteomics in the bone field holds great promise to increase our understanding of protein expression, dynamics, decay, post-translational modifications and signal transduction pathways that regulate bone matrix mineralization. The identification of novel proteins that may be associated with bone matrix mineralization presents important new information toward a better understanding of the precise mechanisms that regulate this process. Moreover, proteomic profiling of osteoblasts, chondrocytes and osteoclasts would greatly enhance our understanding about the molecular pathways regulating bone growth and remodeling. Thus, proteomic profiling provides an efficient method to determine protein candidates and elucidate the signaling transduction pathways that regulate the complex process of bone matrix mineralization.

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