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REVIEW ARTICLE

Subchondral bone proteomics in osteoarthritis: Current status and perspectives

Pok Man Boris Chan [a,1], Lin Zhu [b,1], Chun Yi Wen [a,*], Kwong Yuen Chiu [a,*]

[a] *Department of Orthopaedics and Traumatology, Li Ka Shing Faculty of Medicine, Hong Kong, China*
[b] *Department of Chemistry, The University of Hong Kong, Pokfulam, Hong Kong, China*

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Summary Osteoarthritis (OA) is the most common degenerative joint disorder. OA was conceived as a "wear and tear" problem of articular cartilage, yet there is a lack of treatment options to delay or rescue articular cartilage degeneration once it is established. Actually, the degradation of articular cartilage is related to a complex network of biochemical pathways involving the diffusion of catabolic factors within and between different joint tissues and particularly bone and cartilage. Advanced proteomics technology provides a powerful tool to allow us to build up a library of such factors. Factors that govern the bone-cartilage interplay could be the candidate diagnostic biomarkers and therapeutic targets for OA. Currently, a growing body of proteomic studies has been done to unveil a number of inflammatory cytokines, proteases, and cartilaginous matrix cleavages in the blood serum, synovial fluid, and articular cartilage from OA patients. Little information is available regarding the protein profiles of disturbances at subchondral bone in the pathophysiology of OA. The technical difficulties in protein extraction from tissues particularly bone and quantitative analyses of protein profile are discussed; cellular proteomics of the defective osteoblasts and secretomics for the osteoblasts—chondrocytes crosstalk are proposed to supplement the information obtained from the bone tissue proteomics.

* Corresponding authors. Department of Orthopaedics and Traumatology, L9-12, Lab Block, Li Ka Shing Faculty of Medicine Building, 21 Sassoon Road, Pokfulam, Hong Kong, China.
E-mail addresses: paulwen@hku.hk (C.Y. Wen), pkychiu@hku.hk (K.Y. Chiu).
[1] These two authors contributed equally to this work.

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Introduction

Osteoarthritis (OA) is a prevalent debilitating whole-joint disorder, which commonly afflicts the load-bearing joints such as knees and hips [1]. OA is a major cause for joint pain and disability in elderly people. The hallmark of OA is loss of articular cartilage, which cushions the joint during movement. Yet the integrity of articular cartilage relies on the interplay with other joint tissues, particularly subchondral bone [2]. Given the lack of treatment options to delay or rescue degradation of articular cartilage, bone antiresorptives and anabolics are recently the candidate treatments for OA [3]. Yet the factors or mediators that govern the bone-cartilage interactions in the pathogenesis of OA remain largely unknown.

Proteomics, a large-scale analysis of proteins that involves isolation, purification, and mass spectrometry of proteins of interest, makes it possible to search such factors or mediators in a systemic fashion. Proteomic technologies have been widely adopted in studying various rheumatic diseases [4—10]. Development of high-throughput and high sensitivity mass spectrometry has opened a door to look into the proteins and peptides contained in body fluids and joint tissues [11,12]. Synovial fluid and serum are the frequently studied specimens whereas synovial and cartilaginous tissues were studied in only a handful of studies. To the best of our knowledge, OA subchondral bones have yet to be studied due to some technique challenges in the protein extraction, purification, and identification process. With the advancement of proteomic technologies, it allows us to study the proteins or peptides inside bone that are likely to participate in the interplay between bone and cartilage in the pathogenesis of OA.

To the best of our knowledge, little information is available regarding the challenges and perspectives of subchondral bone and cell proteomics in the context of OA pathophysiology, which is the motivation of this review article.

Tissue proteomics in OA

The past decade has witnessed the values of proteomic technology in identification of biomarkers and therapeutic targets for various arthritis and rheumatic disorders. Proteomics, i.e., establishing a library of proteins and peptides of interest, enables us to delineate the diseased status from their healthy counterparts. Synovial fluid is the most commonly studied specimen in the field of arthritis and rheumatology research. As a dialysate of plasma, synovial fluid contains a much lower concentration of high molecular weight proteins than plasma, and its total amount of proteins is also 30% lower than plasma in physiological conditions [13]. In an inflamed joint, proteins in the blood stream can enter synovial fluid freely due to an increase in blood vessel numbers and permeability. Over 100 inflammation-related proteins such as apolipoproteins, complements, and fibrinogens have already been identified in synovial fluid samples from OA patients [14—16]. Their biochemical functions could be grouped into three dominant pathways: acute phase response signalling, complement pathway, and coagulation pathway. Apolipoproteins might originate from systemic metabolism and complements could be produced locally by synoviocytes. A degraded derivative of complement 3f (C3f) was identified in synovial fluid (SF) of OA patients [15]. C3f, a plasma zinc metalloproteinase, was known as an inflammatory regulator and is related to vascular involvement in another rheumatic disorder — systemic sclerosis [17]. Ligands for toll-like receptors such as hyaluronic acid, fibronectin, and alarmins (S100 proteins) were also detected in OA synovial fluid [18]. They induce macrophages and synoviocytes to produce inflammatory cytokines, mediating the catabolic responses in degenerative process of articular cartilage. Inflammation-related proteins are not only produced in the late-stage, but also in the early stages of OA. This implies that innate immunity might contribute to the onset and progression of OA. Meanwhile, it was not surprising to note a decrease in cysteine proteases inhibitors level in OA synovial fluid [14], which failed to protect aggrecan from degradation. As a consequence, the level of extracellular matrix proteins, e.g., aggrecan and cartilage oligomatrix protein, were significantly increased in OA synovial fluid [9].

Although some meaningful results have been produced, the technical challenges in proteomic studies on OA synovial fluid have to be addressed. Firstly, the abundant proteins in synovial fluid such as various extracellular matrix components and albumins may mask the minutely present proteins, e.g., inflammatory cytokines. Structural proteins could be depleted via immunodepletion or two-dimensional cleanup kits [10,15]. Acetone precipitation, despite being a fairly commonly used technique for proteome study, should not be employed because it lowers the overall protein concentration, including the target proteins [19]. Multiple fractionations involving SDS—polyacrylamide gel electrophoresis (PAGE) for protein level and SCX-Offgel at peptide level could significantly reduce the complexity of the sample [20] and hugely increased the number of newly identified novel proteins. The greatest hurdle to be overcome, however, is that it is very difficult to find a healthy control for OA proteomics study. Usually, synovial fluid samples from rheumatoid arthritis (RA) patients are being compared, which may not expand our understanding in OA.

Synovial fluid is partly produced by synovium, a thin lining in the joint cavity responsible for homeostasis and joint functions. Fibroblast-like synoviocytes is the major cell type in synovium, which can synthesize hyaluronic acid to stabilize water content in synovial fluid and lubricate the articular surfaces during joint movements. Synovial tissues were often investigated in RA, and OA synovium usually serves as a "noninflammatory" control. A transcriptome-proteome combined study on RA and OA synovial tissue demonstrated that many gene expression changes did not coincide at transcription and protein levels, again verifying the need of a more powerful proteome study [8]. To further investigate the physical distribution of proteins, a study involving Matrix-assisted laser desorption/ionization mass spectrometry (MALDI—MS) imaging was performed and this technique involves the use of digital photography of stained histological sections of synovium and MALDI mass spectrometry. Interestingly, the expression of thymosin beta-4, responsible for T-lymphocyte maturation, increased in synovial lining of both RA and OA samples. This finding did