Matrix Biology 39 (2014) 56–66

Contents lists available at ScienceDirect

Matrix Biology

journal homepage: www.elsevier.com/locate/matbio

Mini review

Biomarkers and proteomic analysis of osteoarthritis

Ming-Feng Hsueh [a,c], Patrik Önnerfjord [d], Virginia Byers Kraus [a,b,*]

[a] Duke Molecular Physiology Institute, Duke University School of Medicine, Duke University, Durham, NC, United States
[b] Department of Medicine, Duke University School of Medicine, Duke University, Durham, NC, United States
[c] Department of Pathology, Duke University School of Medicine, Duke University, Durham, NC, United States
[d] Department of Clinical Sciences, Lund University, Lund, Sweden

ARTICLE INFO

Available online 30 August 2014

Keywords:
Biomarker
Proteomics
Osteoarthritis
Cartilage tissue
Cartilage explants
Chondrocytes
Synovial fluid
Serum
Urine
Mass spectrometry

ABSTRACT

Our friend and colleague, Dr. Dick Heinegård, contributed greatly to the understanding of joint tissue biochemistry, the discovery and validation of arthritis-related biomarkers and the establishment of methodology for proteomic studies in osteoarthritis (OA). To date, discovery of OA-related biomarkers has focused on cartilage, synovial fluid and serum. Methods, such as affinity depletion and hyaluronidase treatment have facilitated proteomics discovery research from these sources. Osteoarthritis usually involves multiple joints; this characteristic makes it easier to detect OA with a systemic biomarker but makes it hard to delineate abnormalities of individual affected joints. Although the abundance of cartilage proteins in urine may generally be lower than other tissue/sample sources, the protein composition of urine is much less complex and its collection is non-invasive thereby facilitating the development of patient friendly biomarkers. To date however, relatively few proteomics studies have been conducted in OA urine. Proteomics strategies have identified many proteins that may relate to pathological mechanisms of OA. Further targeted approaches to validate the role of these proteins in OA are needed. Herein we summarize recent proteomic studies related to joint tissues and the cohorts used; a clear understanding of the cohorts is important for this work as we expect that the decisive discoveries of OA-related biomarkers rely on comprehensive phenotyping of healthy non-OA and OA subjects. Besides the common phenotyping criteria that include, gender, age, and body mass index (BMI), it is essential to collect data on symptoms and signs of OA outside the index joints and to bolster this with objective imaging data whenever possible to gain the most precise appreciation of the total burden of disease. Proteomic studies on systemic biospecimens, such as serum and urine, rely on comprehensive phenotyping data to unravel the true meaning of the proteomic results.

Contents

* Corresponding author at: Box 104775, Duke Molecular Physiology Institute, Durham, NC 27701, United States. Tel.: +1 919 681 6652; fax: +1 919 684 8907.
E-mail address: vbk@duke.edu (V.B. Kraus).

http://dx.doi.org/10.1016/j.matbio.2014.08.012

1. Introduction

The investigator for whom this issue is dedicated, Dr. Dick Heinegård, played a pivotal role in the discovery and validation of biomarkers for osteoarthritis (OA). His seminal work, in characterizing the biochemical composition and interaction of components of cartilage, laid the foundation for all the work that has followed in this field. This paper briefly summarizes his contributions to the field and is followed by an update on the results of proteomic analyses performed since 2009 when a comprehensive review of this topic was last published (De Ceuninck and Berenbaum, 2009). Herein we focus on proteomic studies of four different types of biospecimens that are relevant to the study of joint diseases: cartilage, synovial fluid, serum and urine. We dedicate this work to our friend and colleague, Dick Heinegård.

1.1. Contributions to the field

A PubMed search (April 1, 2014) of papers authored by Dr. Dick Heinegård yielded 325 citations; a total of 16% of these were directly related to molecular markers of joint tissues in health and disease, and an additional 48% of citations involved cartilage biochemistry and chondrocyte biology that greatly inform biomarker work. In addition to cartilage biochemistry, he contributed many fine works related to extracellular matrices of other joint tissues and other tissues in the body, including tendon, bone, skin, sclera, cornea, aorta, eye and kidney (Franzen and Heinegård, 1985; Mörgelin et al., 1989; Oldberg et al., 1989; Saxne and Heinegård, 1989; Reinholt et al., 1990). His musculoskeletal work encompassed OA, rheumatoid arthritis (RA), juvenile inflammatory arthritis, polychondritis, reactive arthritis, psoriatic arthritis, and calcium pyrophosphate deposition disease among others (Saxne et al., 1987; Saxne and Heinegård, 1989). A brief summary of a few of his key works and insights are provided below.

For his entire career, Dr. Heinegård was deeply engaged in understanding disease pathogenesis and elucidating the components of various extracellular matrices, and cartilage and bone in particular, and converting what were enigmas into proteins with known structures and functions. This is nicely exemplified in his early creative determination of the substructures of cartilage proteoglycan and link protein; with Wieslander et al., he created tryptic peptide 'maps' from these proteins based on their cross-reactivity to polyclonal antisera developed to specific epitopes within these proteins (Wieslander and Heinegård, 1979). Beginning as early as 1987, with Inerot et al., he characterized the normal variability in structure and composition of the articular cartilage proteoglycans in the hip (Inerot and Heinegård, 1987). In further work with Wiberg et al. (2003) using molecular electron microscopy in combination with immunogold techniques, he was able to reconstitute and visualize collagen VI microfibril complexes *in vitro*; this work showed that the leucine-rich small proteoglycans (biglycan and decorin) together with matrilins form a link between collagen VI microfibrils and the collagen II and aggrecan networks in the cartilage extracellular matrix. This lifelong interest culminated in a recent paper with Önnerfjord et al., comparing hip and knee cartilage that demonstrated that cartilage constituents differ by joint site (Önnerfjord et al., 2012).

With Petersson et al., he showed that serum cartilage oligomeric matrix protein (COMP) was correlated with knee bone scan abnormalities in individuals with knee pain, suggesting that this marker may be a means of evaluating tissue changes in relation to early stages of OA (Petersson et al., 1998). Further work demonstrated that one function for COMP is to influence the organization of collagen fibrils, thereby contributing to tissue structure. He demonstrated that COMP interacted via its C-terminal globular domain to collagens I and II in the presence of Zn^{2+} and Ni^{2+} but not Ca^{2+}, Mg^{2+}, and Mn^{2+}. Electron microscopy with Rosenberg et al., showed that the interaction occurred at four defined sites on the collagen molecules (Rosenberg et al., 1998).

He also used COMP to gain insights into efficacy of interventions in OA. Working with Sharif et al., he showed that serum COMP increased significantly during the first year of follow-up in patients with progressive OA but not in non-progressors (Sharif et al., 1995). With Joosten et al., he observed synergistic effects of combined treatment with low dose prednisolone, IL-10 and IL-4 on disease activity of collagen induced arthritis reflected in reduced cartilage degradation based on serum COMP concentrations (Joosten et al., 1999a; Joosten et al., 1999c). In further work with Joosten et al., serum COMP also provided insight into the tissue target of biologic treatments of collagen induced arthritis; although both soluble TNF binding protein and anti-IL-1 treatment ameliorated disease activity, only anti-IL-1 treatment normalized COMP levels supporting the histological finding that anti-IL1 decreased cartilage destruction while anti-TNF blocked synovitis (Joosten et al., 1999b).

He monitored other matrix molecules as biomarkers to gain further insights into efficacy of interventions. In a study paradigm that was novel and instructive, even today, with Saxne et al. he evaluated cartilage metabolism in arthritis through measurement of proteoglycan in synovial fluid before and after intra-articular injection with a glucocorticoid (Saxne et al., 1986). He established the stability of the proteoglycan measure in samples withdrawn 5 days apart; then the patients were treated with local injections of glucocorticoids that were observed to significantly reduce the proteoglycan concentration in the joint fluid. This was one of the earliest *in vivo* demonstrations that quantification of proteoglycans in synovial fluid appears to have the potential for monitoring the effects of therapy on cartilage metabolism.

With Lorenzo et al. (2004), two key observations were made: that cartilage undergoes metabolic alterations very early in the disease process, even before there is overt fibrillation of the tissue; and notably, in contrast to traditional teaching suggesting minimal or no repair (in the case of collagen II), attempts to repair or replace the extracellular matrix in knee OA were evident based on aggrecan synthesis and increases in cartilage oligomeric matrix protein (COMP), fibronectin, and cartilage intermediate layer protein (CILP) (early events in the process) and collagen synthesis (late event in the process). Importantly, they drew attention to the challenge we have yet to overcome even today, the ability to define and distinguish early from late OA; they defined early OA as the absence of an OA clinical history but the presence of macroscopic lesions in the joint, while acknowledging that "the disease is only recognized in its late stage by clinical and radiological criteria".

In novel work with Sjöberg et al. (2005) he was among the earliest investigators to recognize the ability of components of the extracellular matrix, including the small leucine-rich repeat proteins (SLRPs) fibromodulin, osteoadherin, and chondroadherin, to activate the complement system that forms the core of the innate immune system. With Happonen et al. (2009) he interestingly found that fibromodulin, osteoadherin, and chondroadherin also bound the complement inhibitor C4BP; although not interfering with the ability of C4BP to inhibit complement, this binding apparently sequestered the SLRPs and thereby modulated their pro-inflammatory effects. They confirmed published data (Groeneveld et al., 2005) that decorin and biglycan bound C1-complex recognition protein C1q but did not activate complement (Sjöberg et al., 2009). In the same study, they also showed that lumican had similar properties but with lower affinity for C1q. Moreover, with Happonen et al. (2010) he showed that COMP can activate one complement pathway at the same time as it has the potential to inhibit another. He intuited that the "net outcome of these interactions is most likely determined by the type of released COMP fragments, which may be disease specific". With Kalchishkova et al. (2011), the NC4 domain of collagen IX was shown to inhibit complement by preventing complement C9 polymerization and enhancing cofactor activities of the major soluble complement inhibitors C4BP and Factor H. With Happonen et al. (2012b), compared with healthy controls he found elevated levels of COMP-C3b complexes in the circulation of patients with several rheumatologic diseases, including RA, OA, reactive arthritis, psoriatic arthritis, systemic lupus erythematosus, ankylosing spondylitis, and systemic sclerosis. COMP-C3b correlated with several measures reflecting