Applications of proteomics to osteoarthritis, a musculoskeletal disease character...

Health & Medicine

The Global Challenge of Musculoskeletal Diseases Characterized and Exacerbated by Aging

The incidence of age-related diseases is rising, seriously affecting the health of millions of people around the world. According to the United Nations (UN) 1 and the World Health Organization (WHO) 2 musculoskeletal, rheumatic, and arthritic conditions are leading causes of morbidity and disability throughout the world, giving rise to enormous healthcare expenditures and loss of work (Woolf and Pfleger, 2003 ; source: http://www. arthritis. org/) 3 , 4 . Many types of rheumatic diseases and arthritic conditions are essentially age-related " inflammatory" disorders where the inflammation facilitates disease progression. The term " arthritis" characterizes a group of conditions involving inflammatory damage to synovial joints (Di Paola and Cuzzocrea, 2008). Arthritis literally means inflammation (*itis*) of the joints (*arthr*). It involves pain, redness, heat, swelling, and other harmful effects of inflammation within the joint. There are over 200 different forms of arthritis. However, the most common and important form of arthritis is osteoarthritis (OA), also known as osteoarthrosis or degenerative joint disease (DJD). OA is the most prevalent of the chronic diseases affecting the elderly (Aigner et al., 2004). The majority of the population over 65 years of age demonstrate radiographic evidence of OA in at least one joint. Although OA is rare in people under 40, it becomes much more common with age. More than 20 million Americans are estimated to have OA 5 . A 2005 study in the USA estimated that OA is one of the top five causes of disability amongst non-hospitalized adults [source: Center for Disease Control (CDC 6), USA]. In 2006 it was estimated that around 35 million to 40 million Europeans suffer

from OA and nearly 25% of people aged 60 and above suffer from OA induced disability. It is also anticipated that by the year 2030, 20% of adults will have developed OA in Western Europe and North America. Therefore, OA is expected to place a heavy economic burden on healthcare systems and community services throughout the world. The risk factors for OA are well known and include age, overweight/obesity, underlying metabolic or endocrine disease, genetics, and joint trauma (Lotz and Kraus, 2010). With increasing life expectancy, growth in the elderly population and an alarming escalation of chronic, inflammatory, and age-related conditions (such as OA), there is increased demand for new treatments and preventative approaches.

Articular Cartilage Structure and Function

Articular cartilage is the main tissue involved in OA. It is a mechanically unique and resilient connective tissue responsible for load-bearing and low-friction movement in the synovial joints of all vertebrates (Buckwalter et al., 2005). Cartilage is avascular and as a consequence it has a very limited capacity for intrinsic repair (Brittberg, 1999 ; Tew et al., 2001). It highly prone to structural degradation making it particularly difficult to restore once it is damaged or lost. The extracellular matrix (ECM) of cartilage gives the tissue resilience and elasticity. The ECM consists of three classes of molecules: collagens, aggregating proteoglycans, and non-collagenous proteins. Type II, IX, and XI collagens form a fibrillar framework of macromolecules that give the tissue form, tensile stiffness, and mechanical strength (Buckwalter and Mankin, 1998b ; Eyre, 2004). Large aggregating proteoglycans (predominantly aggrecan) allow cartilage to swell and resist compressive forces (Hardingham and Fosang, 1992 ; Kuettner, 1992). Small

proteoglycans including decorin, biglycan, and fibromodulin, bind to other matrix macromolecules and help to stabilize the ECM. Other collagenous and non-collagenous macromolecules present within the ECM perform a variety of structural and informational roles, facilitate cell–cell and cell-matrix interactions, and bind growth factors (Hardingham and Fosang, 1992 ; Feng et al., 2006). The chondrocyte is the only cell type present in articular cartilage (Archer and Francis-West, 2003). During embryonic development chondrocytes synthesize a cartilaginous template for endochondral ossification and skeletal development and in postnatal life they maintain the ECM by regulating the turnover of matrix components in response to biomechanical, biochemical, and endocrine signals (Goldring and Marcu, 2009). Chondrocytes actively synthesize new ECM components as well as the proteolytic enzymes such as matrix metalloproteinases (MMPs), a disintegrin, and metalloproteinase (ADAMs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) are responsible for tissue remodeling during development. These enzymes are also involved in the catabolic breakdown of cartilage in OA (Aigner et al., 2006).

Cartilage Degradation in Osteoarthritis

Osteoarthritis is a degenerative disease that involves joint inflammation, bone remodeling, and catabolic destruction of the articular cartilage component (Goldring and Goldring, 2007 ; Samuels et al., 2008). In OA there is an imbalance between the synthesis and degradation of ECM macromolecules (Felson, 2004). This can be due to increased enzymatic activity of MMPs (Okada et al., 1992), and pro-inflammatory mediators such as cytokines (Goldring and Goldring, 2004), prostaglandins, and nitric oxide

(Goldring and Berenbaum, 2004), coupled with the reduced anabolic capacity of chondrocytes (Aigner et al., 1997) and the tissue's inherently poor reparative capacity due to its avascular nature (Archer and Francis-West, 2003). Consequently OA is characterized by the loss of structural constituents from the ECM. The degradation and release of proteins and glycoproteins from cartilage in OA can vary according to the stage of the disease process. For example, elevated serum cartilage oligomeric matrix protein (COMP) is correlated with the presence of OA and disease severity (Clark et al., 1999).

Aging and Osteoarthritis

Aging is a major contributor to musculoskeletal degeneration and the development of OA (Hamerman, 1998 ; Lotz and Carames, 2011). Age-related changes in articular cartilage contribute to the development and progression of OA. Although the degeneration of articular cartilage is not simply the result of aging and mechanical wear, aging nevertheless modifies the articular joint including cartilage, subchondral bone, muscle, soft tissues, synovial membrane, and synovial fluid (Buckwalter and Mankin, 1998a ; Hamerman, 1998). Although older age is the greatest risk factor for OA, OA is not an inevitable consequence of growing old (Shane Anderson and Loeser, 2010). The mechanisms for the link between aging and OA are incompletely understood. Cell stress and oxidative damage contribute to chronic inflammation that promotes age-related diseases. In OA this results in senescence-associated secretory phenotype, which has many of the characteristics of an osteoarthritic chondrocyte in terms of the cytokines, chemokines, and proteases produced (Loeser, 2011).

Biomarkers of Osteoarthritis

A major focus of clinical research in recent years has been the identification of new disease markers that can facilitate early diagnosis and optimize individualized treatments. Such markers can also facilitate the drug discovery process by reducing the high levels of attrition in clinical trials. A biomarker is classically defined as a biochemical entity that is used to measure the progress of a disease or the effects of treatment on clinical outcome. Biochemical markers can be measured in blood, serum, and urine or a variety of other body fluids and tissues. The National Cancer Institute (NCI) 7 defines a biomarker as “ a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease,” and the terms “ molecular markers” or “ signature molecules” have also been used to describe such markers. The term biomarker is all encompassing and can include proteins, protein fragments, metabolites, carbohydrates, nucleic acids (RNA and DNA), cellular features, and images.

Osteoarthritis is unambiguously diagnosed when it is “ detected” by the best available test. Thus far the best test for this purpose has been radiography, the so-called “ gold-standard.” This process also requires clinical signs in the patient, which often occur well into the progression of the disease. However, there is often early, pre-clinical evidence of disease provided by various biomarkers, which if detected, may facilitate earlier diagnosis and treatment. Such an approach is particularly pertinent in the case of OA, a disease often characterized by a prolonged pre-clinical “ molecular” phase, a “ pre-radiographic” phase, and a “ recalcitrant radiographic” phase by which time

there are structural changes to joints along with pain and loss of function. Biomarkers have the potential to provide an early warning of joint degeneration which could prompt earlier, more targeted treatment to prevent the tissue destruction that results in the characteristic chronic disability associated with OA. In this context, biomarkers could make a significant contribution to the early diagnosis of OA, as well as informing key aspects of disease prognosis, monitoring, and therapy.

The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) established the Osteoarthritis Biomarkers Network 8 to develop and validate standardized, sensitive biomarker assays in blood and urine to facilitate the diagnosis of the pre-radiologic stage of OA in humans and in animal models. Such markers can help us understand the biological processes involved in disease progression and allow us to monitor the effects of surgical or pharmacological treatment, thus accelerating the pace of drug discovery. Such biomarkers could also potentially be used to identify patients at increased risk of developing OA. Existing biomarkers of OA have major limitations: they do not “ flag” the pre-radiographic phase of the disease; they are not specific for the various stages of OA, and in some cases, may not even be specific for OA.

Considering these challenges, the Osteoarthritis Research Society International (OARSI) 9 and the US Food and Drug Administration (FDA) 10 have recently established a new OA biomarkers working group, which has proposed the division of potential markers into two major groups: the so-called soluble or “ wet” biomarkers, which typically reflect a modulation in an

endogenous substance in body fluids such blood, serum, plasma, urine, or synovial fluid); and the “ dry” biomarkers, which usually consist of visual analog scales, performed tasks, or images of joints (Kraus et al., 2011).

Therefore, the ability to detect biomarkers of cartilage degradation and/or inflammation in biological samples, such as serum, urine, or synovial fluid, may enable clinicians to diagnose sub-clinical OA as well as determining the disease stage in both human and companion animals. Identifying these biomarkers will also aid drug discovery and drug safety/efficacy monitoring in patients and in animal models. Using combinations of biomarkers may be more effective in achieving these goals, thus having a panel of biomarkers will help researchers and the pharmaceutical industry to monitor disease progression as well as to assess responses to treatment in experimental models of OA (Rousseau and Delmas, 2007 ; Williams, 2009).

Systems Biology and Proteomic Approaches for the Discovery of Osteoarthritis Biomarkers

Systems biology is increasingly applied in orthopedics and rheumatology to cartilage and synovium in arthritis. These techniques include genomics, transcriptomics, proteomics, metabolomics, glycomics, and bioinformatics and can be applied to the study of cartilage, synovium, synovial fluid, and even blood (serum) or urine from OA patients. Proteomics involves the application of specialized analytical techniques that allow the evaluation of the protein composition of tissues, cells, and culture supernatants. Proteomics is being increasingly applied in basic cartilage biology (Polacek et al., 2010) and OA research (Ruiz-Romero et al., 2010). Characterization of cell lysates from isolated chondrocytes has yielded valuable information

regarding the intracellular proteins of the chondrocyte proteome, and paved the way for future studies on cartilage pathologies such as OA (Ruiz-Romero et al., 2005 ; Ruiz-Romero and Blanco, 2010). Studies of soluble proteins in cartilage tissue from OA patients has increased the knowledge of the proteins contained within the ECM of diseased versus normal tissue (Wu et al., 2007). A number of papers have reported on proteins secreted from the cartilage ECM in response to pathological insults such as interleukin (IL)-1α and all-trans-retinoic acid (Wilson et al., 2008a , b ; Ruiz-Romero and Blanco, 2010), IL-1β and TNF-α (Cillero-Pastor et al., 2010) and mechanical compression (Stevens et al., 2008 ; Zhang and Wang, 2009 ; Li et al., 2010). Identifying proteins released from cartilage has the potential to give an indication of disease biomarkers likely to be present in the synovial fluid or blood of patients in the early stages of OA.

Relevance of Biomarkers and Proteomic Techniques to “ Physiology and Pathophysiology of Musculoskeletal Aging”

Understanding healthy aging is a key research priority, along with a better understanding of the pathophysiology of aging that occurs in a number of age-related diseases, such as arthritis. By gaining a better understanding of healthy musculoskeletal aging we can provide better care and new therapies for common musculoskeletal problems. “ Physiology and Pathophysiology of Musculoskeletal Aging” is a Research Topic that is intended to bring together basic researchers and clinicians working in the broad area of musculoskeletal aging. The topic includes mechanisms of healthy aging in tissues of the musculoskeletal system (i. e., skeletal muscle, articular cartilage, subchondral bone, tendon, and ligament).

The discovery and validation for biomarkers of OA has accelerated significantly as our understanding of joint tissue molecules and their complex interactions have increased (Kraus, 2005). One of the main drivers in this context has been the urgent need for improved OA “ outcome measures” in clinical trials (Kraus, 2005 ; Hunter et al., 2010). In particular there is a pressing need for new biomarkers that indicate early responses of the joint cartilage to degeneration that will be useful in detecting early, pre-radiographic changes. Novel markers that characterize the status and prognosis of OA, and that can be used to monitor response to therapy are also required (Mobasheri and Henrotin, 2010). Current “ omics-based” research aims to develop an “ analytical toolbox” which is hoped will contribute to the clinical development process (Bay-Jensen et al., 2010 ; Qvist et al., 2010). Combinations of existing biomarkers may improve their prognostic accuracy and help identify at-risk patients (Williams, 2009). The challenge is to use proteomics and other “ omics-based” technologies in order to identify sensitive and reliable pre-radiographic biomarkers that can be accurately and reproducibly measured in body fluids. Biomarkers that “ flag” early stage OA will be particularly useful in curbing disease progression by identifying patients that would benefit from early therapeutic intervention.

In this Research Topic Gharbi and co-workers (Gharbi et al., 2011) review the applications of proteomic techniques for studying OA. Their aim is to improve our understanding of the physiopathology of the disease its underlying mechanisms and to discover disease-specific biomarkers and identify new therapeutic targets. This timely and focused review summarizes

the currently available data regarding proteomic techniques and their applications to OA research. The authors discuss technical limitations and solutions to real and practical problems including sample preparation. Although proteomics has many potential applications in this area, there are technical challenges that still remain. This elegant and original article highlights the major issues facing researchers in this area.

Conflict of Interest Statement

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Footnotes

1. ^ http://www. un. org/
2. ^ http://www. who. int/en/
3. ^ http://www. who. int/healthinfo/statistics/bod_osteoarthritis. pdf
4. ^ http://whqlibdoc. who. int/bulletin/2003/Vol81-No9/bulletin_2003_81(9)_630. pdf

5. ^ http://www. niams. nih. gov/
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