Osteoarthritis and Cartilage 25 (2017) 1199–1209

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

Chondrocyte secretome: a source of novel insights and exploratory biomarkers of osteoarthritis

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ARTICLE INFO

Article history:
Received 30 November 2016
Accepted 14 February 2017

Keywords:
Cartilage
Chondrocyte
Osteoarthritis
Biomarker
Proteomics
Secretome

SUMMARY

The extracellular matrix (ECM) of articular cartilage is comprised of complex networks of proteins and glycoproteins, all of which are expressed by its resident cell, the chondrocyte. Cartilage is a unique tissue given its complexity and ability to resist repeated load and deformation. The mechanisms by which articular cartilage maintains its integrity throughout our lifetime is not fully understood, however there are numerous regulatory pathways known to govern ECM turnover in response to mechanical stimuli. To further our understanding of this field, we envision that proteomic analysis of the secretome will provide information on how the chondrocyte remodels the surrounding ECM in response to load, in addition to providing information on the metabolic state of the cell. In this review, we attempt to summarize the recent mass spectrometry-based proteomic discoveries in healthy and diseased cartilage and chondrocytes, to facilitate the discovery of novel biomarkers linked to degenerative pathologies, such as osteoarthritis (OA).

Introduction

To gain a deeper understanding of the mechanisms that drive osteoarthritis (OA), it is important to appreciate the underlying biology of healthy and diseased joint tissues. Of interest is the pathophysiology of articular cartilage, and the processes that govern synthesis and organisation of extracellular matrix (ECM) components secreted by chondrocytes into the pericellular milieu. The chondrocyte is the unique resident cell of articular cartilage and thus solely responsible for ECM composition and regulation. Chondrocyte metabolism is influenced by its micro-environment, and in return influences ECM composition, organization and ultimately the mechanical resilience of cartilage[1–3]. As such, chondrocytes play a key role in ECM remodelling in physiological and pathological conditions[4].

It is commonly established that healthy articular chondrocytes change into different phenotypes as OA develops and progresses:

(i) A catabolic phenotype develops, associated with an increase in proteolytic enzymes and reactive oxygen/nitrogen species,

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http://dx.doi.org/10.1016/j.joca.2017.02.797
1063-4584/

in response to mechanical stress and inflammatory cytokines, tumour necrosis factor (TNF)-α and interleukin (IL)-1β, leading to further ECM degradation.

(ii) An anabolic phenotype emerges that is associated with regeneration of the ECM, including increased collagen type II and proteoglycan expression regulated by growth factors (transforming growth factor (TGF)-β, bone morphogenetic protein (BMP)s and insulin growth factor (IGF)-I), expressed either by the surrounding joint tissue or by the chondrocytes themselves.

(iii) A hypertrophic phenotype develops, manifesting in expression of type X collagen and induction of apoptosis, ultimately resulting in osteophyte formation.

(iv) A fibroblastic-like phenotype with an increased number of dedifferentiated chondrocyte and expression of type I collagen.

(v) Lastly, a chondroblastic phenotype emerges with expression of foetal type IIA collagen, type III collagen and early/late differentiation markers[5].

The specific phenotype that any individual chondrocyte exhibits is dependent on the zone in which the chondrocyte is situated and the stage of OA progression. In the upper zone, cellular proliferation and hypertrophy are observed, whereas the mid and deep zones display increased expression of type II collagen[6]. As OA progresses, cartilage is lost and chondrocytes undergo senescence, due to a combination of replicative exhaustion and oxidative stress[7].

Eventually, chondrocytes will undergo apoptosis, and the articular cartilage will be destroyed. The ultimate goal of mass spectrometry-based proteomics strategies is the identification of a specific tissue-derived secretome that is unique to the diseased chondrocyte and surrounding ECM and is able to distinguish between healthy and diseased cartilage.

Because protein expression is dependent on environmental conditions, the secretome is highly dynamic in composition and turnover. In 2010, Agrawal *et al.* suggested defining the secretome as "the global group of secreted proteins into the extracellular space by a cell, tissue, organ, or organism at any given time and conditions through known and unknown secretory mechanisms involving constitutive and regulated secretory organelles"[8]. In this narrative review, we only considered the chondrocyte (or cartilage) as the source of secreted proteins, which have been uncovered by state-of-the-art mass spectrometry-based proteomics techniques.

Proteomic techniques include different methods, all relying on the separation of proteins and their further analysis using either gel-based (Two-dimensional electrophoresis) or gel-free methods. Protein separation methods are coupled to a mass spectrometer for identification of sequence by mass spectrometry[9,10]. In differential analysis, the peptides may be marked with stable isotope at various stages of the analysis process, or Label-free methods could be performed. Several modes of analysis are available in mass spectrometry[10]. They differ markedly by the ionization source of the sample. The main sources used in proteomic analysis are matrix-assisted laser desorption/ionization (MALDI) and surface-enhancer laser desorption/ionization (SELDI)[10,11]. These techniques allow a soft ionization of molecules without excessive fragmentation[9,11].

More than the total tissue protein extract, it is expected that well-defined protein fractions such as the secretome could be a source of novel OA biomarkers, with the potential to predict disease severity and monitor progression.

Materials and methods

A literature search was performed in Pubmed/Medline and Scopus, identifying articles published between January 2004 and March 2016. Keywords used in 'Any fields' were; (chondrocyte OR cartilage) AND secretome (19 relevant papers out of 38 found), or (chondrocyte OR cartilage) AND (proteomic OR mass spectrometry) (65 articles relevant papers out of 290 found). Only results from mass spectrometry-based studies were included in this article. The review has considered all species, even if the majority of studies have been performed on human source of chondrocytes or cartilage. Only research articles published in English were included. Supplementary files of all papers were analysed and included in this review.

Chondrocyte secretome

Recent mass spectrometry-based proteomic studies have identified several proteins which form the chondrocyte secretome. In this part of the review, we focused on proteins identified either directly in the secretome of cartilage explants[12–20] or chondrocytes cultures[18,21–31]), both of which are listed in Table I and illustrated in Fig. 1. We further complete this list with proteins recently identified by proteomic analysis performed directly on fresh chondrocytes or cartilage tissue[32–36] (sometimes de-cellularized[37]) whereby different locations in the joint were compared, or healthy joint tissues were compared with OA tissues. The characteristics of all these studies are summarized in Table II. Proteins have been classified in different sections: ECM proteins, cytokines and growth factors, enzymes and miscellaneous.

ECM proteins

As expected, the most abundant ECM proteins produced by chondrocytes, and detected by proteomic analysis, are collagens and proteoglycans, Table I. Thirteen collagens are found in the chondrocytes secretome, of which type II, VI and XII are the most abundant[35]. Collagen type XII is also known to interact with other cartilage elements such as cartilage oligomeric matrix protein (COMP), decorin and fibromodulin[38]. Collagen type II and VI levels are increased in the secretome of chondrocytes taken from the medial condyle of patients with early OA (Mankin score 0–3), compared with samples taken from patients with severe OA (Mankin score 5–10)[25].

Beside the collagens, other ECM proteins found in high abundance in the chondrocyte secretome include; aggrecan, HPLN-1 (proteoglycan link protein), biglycan, COMP, fibronectin, prolargin (Proline-arginine-rich end leucine-rich repeat protein (PRELP)), matrilin-3, cartilage acidic protein-1 (CRTAC1 or ASPIC), latent-transforming growth factor beta-binding protein-1 (LTBP1), extracellular matrix protein-1 (ECM-1), tenascin, lubricin and chitinase-3-like protein 1 (CHI3L1), also known as YKL-40). CHI3L1 was found at lower levels in cartilage explants treated with IL-1β compared to controls[12]. CHI3L1, is a biomarker of OA found in synovial fluid and serum[39], and plays a role in tissue remodelling and inflammation. The concentration of CHI3L1 in OA synovial fluid positively correlates with levels of matrix metalloproteinase (MMP)-1, MMP-3, IL-6 and IL-17.

IL-6 and IL-17 enhanced CHI3L1 production in human primary chondrocyte cultures[40] and CHI3L1 serum concentration positively correlated with osteophyte size in OA patients[41]. This protein is more abundant in knee compared to hip cartilage[37] and in OA