Acta Histochemica 116 (2014) 965–972

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Lubricin expression in human osteoarthritic knee meniscus and synovial fluid: A morphological, immunohistochemical and biochemical study

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ARTICLE INFO

Article history:
Received 23 March 2014
Received in revised form 24 March 2014
Accepted 28 March 2014

Keywords:
Knee menisci
Osteoarthritis
Fibrocartilage
Lubricin
Immunohistochemistry
Western blotting

ABSTRACT

The purpose of this study was to investigate the expression of lubricin, the product of the human PRG4 (proteoglycan 4) gene, in menisci and synovial fluid from normal donors and patients with osteoarthritis (OA), using a combination of histology, immunohistochemistry, ELISA and Western blotting analysis, to provide further insight on the role of this protein in the progression of OA and pathological processes in the meniscus. Lubricin expression was studied in samples from 40 patients and in 9 normal donors after arthroscopic partial meniscectomy. Histological analysis confirmed normal microanatomy and the absence of structural changes in control samples. Menisci derived from OA patients showed evidence of structural alterations, fibrillations and clefts. Immunohistochemical analysis revealed very strong lubricin immunostaining in normal menisci in contrast to weak/moderate staining seen in osteoarthritic menisci. Quantitative ELISA and Western blot analysis confirmed the above results. The findings of this study support the notion that changes in lubricin expression and boundary-lubricating ability of cartilage is followed by the development of OA. This study could provide the biological foundation for the development of novel therapeutic treatments, to be applied before the surgery, for the prevention of post-traumatic cartilage damage.

Introduction

Knee osteoarthritis (OA) is characterized by progressive alterations in joint structures and low-grade inflammation (Berenbaum, 2013). OA is characterized as joint failure due to progressive changes in several musculoskeletal components that include, but are not limited to, articular cartilage (Berenbaum, 2013). Other joint structures, such as the menisci are altered in OA, but have not been as extensively studied as the articular cartilage (Loeser, 2011). In this study special attention was focused on the expression of lubricin in the meniscus, after partial meniscectomy, and in synovial fluid (SF) from OA patients in comparison to normal donors. Partial meniscectomy is a routine surgical procedure that is frequently carried out following damage to the meniscus. Before the introduction of arthroscopy, broken menisci were removed completely with grave consequences for joint articulation and biomechanics, facilitating subsequent progression of the arthritic knee (Musumeci et al., 2012). Today surgical procedures attempt to save as much meniscal tissue as possible, in order to preserve at least a component of its biomechanical function in the knee.

Abbreviations: ACL, anterior cruciate ligament; BSA, bovine serum albumin; DAB, 3,3′-diaminobenzidine; ECL, enhanced chemiluminescence; ES, extent score; H&E, hematoxylin and eosin; HA, hyaluronic acid; IHC, immunohistochemistry; IS, intensity of staining; LSAB, labeled streptavidin antibody; MRI, magnetic resonance imaging; OA, osteoarthritis; ORT, estrogen replacement therapy; PAS, periodic acid-Schiff; PBS, phosphate buffered saline; RA, rheumatoid arthritis; SDS, sodium dodecyl sulphate; SF, synovial fluid; SZP, superficial zone protein; TMJ, temporomandibular joint; TTBS, Tween-Tris-buffered saline.

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http://dx.doi.org/10.1016/j.acthis.2014.03.011
0065-1281/

Increasing evidence suggests that the meniscus may not represent a passive by-stander in the OA processes. Knee menisci are two semilunar biconcave disks (lateral and medial) that reside within the medial and lateral tibio-femoral articulations. They are divided into inner, middle and outer rim (Musumeci et al., 2013a). The inner rim is the most delicate part, because it is not vascularized. The lateral meniscus has the form of an almost complete circle and adheres to the two cruciate ligaments (Musumeci et al., 2013a). The medial meniscus has the form of a half moon and is more extensive than lateral, with its extremities adhering to anterior and posterior intercondylar areas. Between the two menisci, the medial meniscus is more subject to trauma, because it is less mobile than the lateral for the presence of the semimembranosus tendon, but also because usually we tend to have a slight valgus during gait (Musumeci et al., 2013a). Microscopically, the meniscus is composed of dense fibrocartilage, sparsely populated with cells called fibrochondrocytes because they exhibit characteristics of both fibroblasts and chondrocytes (Galanti et al., 2013). The meniscus plays an important role in the complex biomechanics of the knee joint. It is involved in load bearing, load transmission, shock absorption, joint stability, joint lubrication and joint congruity. Its loss results in higher peak stresses exerted on cartilage, and eventually in cartilage degeneration and OA (Musumeci et al., 2012). Lubricin, also known as superficial zone protein (SZP), is a chondroprotective, mucinous glycoprotein, the product of the proteoglycan 4 (PRG4) gene (Musumeci et al., 2013b). It has been found in several tissues including the synovial membranes and synovial fluid (SF) (Elsaid et al., 2005), the superficial zone of articular cartilage (Schumacher et al., 1994), tendon (Rees et al., 2002), ligament (Leonardi et al., 2012a), disk (Leonardi et al., 2012b,c) and meniscus (Schumacher et al., 2005; Young et al., 2006; Zhang et al., 2011; Musumeci et al., 2013b). The essential role of lubricin is maintaining joint integrity. Lubricin was originally identified as a lubricating glycoprotein present in SF, specifically synthesized and expressed by articular chondrocytes of the superficial zone and it is recognized to play a major protective role in preventing cartilage wear and synovial cell adhesion and proliferation and reducing the coefficient of friction of the articular cartilage surface (Musumeci et al., 2011a,b; Loeser, 2013). Lubricin is important for articular joint physiology, and the loss of lubricin accumulation may be involved in the pathology of OA. Previous studies demonstrated that important down-regulation of lubricin, as well as of other proteoglycans and synovial fluid biomarkers, develops in the human knee meniscus and anterior cruciate ligament (ACL) immediately after acute joint injury, underlining its involvement in the articular damage (Catterall et al., 2010; Musumeci et al., 2013b). A study in an animal model of OA demonstrated that recombinant lubricin improves the chondroprotection, which suggests that recombinant lubricin molecules may potentially be used in new approaches for the treatment of OA and associated cartilage disorders (Flannery et al., 2009). The purpose of our study was to investigate, in human patients, the expression of lubricin in menisci and synovial fluid from normal donors and patients with OA, using histology, immunohistochemistry, ELISA and Western blot analysis, to provide insights into the role of this protein in the progression of OA-related pathological processes in the meniscus.

Materials and methods

Patients

Knee menisci were obtained from 40 patients, with OA, with similar height 163.5 ± 7.9 cm (range 150–175) and weight 62.2 ± 9.8 kg (range 50–96), 25 males and 15 females, who underwent isolated arthroscopic partial, medial or lateral, meniscectomy (28 and 12, respectively) performed at the Orthopaedic Surgery Unit, Fondazione GB Morgagni, Catania, Italy. Informed consent was obtained from each patient; the research was approved by the Local Medical Ethical Committee and conformed to the ethical guidelines of the Declaration of Helsinki. Surgery was performed because of the pain and functional impairment. The median age of these patients was 68 (range 51–76 years). Subjects with ligament injury, articular cartilage lesions or combined, lateral and medial, meniscal pathologies were excluded to allow a more accurate assessment of the effects of partial meniscectomy alone. The patients occasionally took non-steroidal anti-inflammatory drugs (NSAIDs), therapeutic ultrasound treatment and terminal isometric exercises. The females in the study did not take estrogen replacement therapy (ORT) that may influence the physiological homeostasis of the joint. The pre-operative examinations included physical examination, X-ray imaging and magnetic resonance imaging (MRI). According to the Kellgren and Lawrence classification, patients had grade 2 (definite osteophytes, definite narrowing of joint space) or grade 3 (moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour) OA of the knee with definite osteophyte and unimpaired joint space and/or moderate diminution of joint space. All the subjects presented a correctable alignment with high tibia osteotomy. Menisci used for the controls were obtained from nine patients with similar height 164.2 ± 7.3 cm (range 157–173) and weight 63.4 ± 8.9 kg (range 53–80), 5 males and 4 females, without any history of primary or secondary arthritis, from the Orthopedic Surgery Unit, Fondazione GB Morgagni, Catania, Italy, under an approved Institutional Review Board protocol that conformed to the ethical guidelines of the Declaration of Helsinki and after the informed consent obtained from the patients. The median age of these patients was 60 (range 45–66 years). Knee menisci were partial removed by arthroscopy about three months after the ACL rupture following a sport traumatic event (no acute injury). Macroscopic and microscopic examination of the meniscus showed no signs of degenerative or inflammatory joint disease.

Histology and histochemistry

Samples were rinsed in phosphate buffered saline (PBS), fixed in 10% buffered formalin as previously described (Musumeci et al., 2013c). After an overnight wash, specimens were dehydrated in graded ethanol, cleared in xylene and paraffin-embedded, preserving their anatomical orientation. Sections (4–5 μm in thickness) were cut from paraffin blocks using a microtome, mounted on polylysine-coated slides and stored at room temperature. The sections were stained with hematoxylin and eosin (H&E) for general cell identification and for the presence or absence of structural alterations. Alcian blue (pH 2.5) and periodic acid-Schiff (PAS) staining was used to assess synthesis of sulfated glycosaminoglycan (GAG) containing proteoglycans (assessment was made on the intensity of staining). The sections were examined with a Zeiss Axioplan light microscope (Carl Zeiss, Oberkochen, Germany) and photomicrographs were captured using a digital camera (AxioCam MRc5, Carl Zeiss, Oberkochen, Germany).

Immunohistochemistry (IHC)

For immunohistochemical analysis, fibrocartilage tissue was processed as previously described (Loreto et al., 2013). Briefly, the slides were dewaxed in xylene, hydrated using graded ethanols and incubated for 30 min in 0.3% H_2O_2/methanol to quench endogenous peroxidase activity before being rinsed for 20 min with phosphate-buffered saline (PBS; Bio-Optica, Milan, Italy). The sections were heated (3× 5 min) in capped polypropylene slide-holders with citrate buffer (10 mM citric acid, 0.05% Tween 20, pH