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Quantitative proteomics at different depths in human articular cartilage reveals unique patterns of protein distribution

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ABSTRACT

The articular cartilage of synovial joints ensures friction-free mobility and attenuates mechanical impact on the joint during movement. These functions are mediated by the complex network of extracellular molecules characteristic for articular cartilage. Zonal differences in the extracellular matrix (ECM) are well recognized. However, knowledge about the precise molecular composition in the different zones remains limited. In the present study, we investigated the distribution of ECM molecules along the surface-to-bone axis, using quantitative non-targeted as well as targeted proteomics.\

In a discovery approach, iTRAQ mass spectrometry was used to identify all extractable ECM proteins in the different layers of a human lateral tibial plateau full thickness cartilage sample. A targeted MRM mass spectrometry approach was then applied to verify these findings and to extend the analysis to four medial tibial plateau samples.

In the lateral tibial plateau sample, the unique distribution patterns of 70 ECM proteins were identified, revealing groups of proteins with a preferential distribution to the superficial, intermediate or deep regions of articular cartilage. The detailed analysis of selected 29 proteins confirmed these findings and revealed similar distribution patterns in the four medial tibial plateau samples.

The results of this study allow, for the first time, an overview of the zonal distribution of a broad range of cartilage ECM proteins and open up further investigations of the functional roles of matrix proteins in the different zones of articular cartilage in health and disease.

1. Introduction

Articular cartilage is a highly specialized tissue that serves to facilitate joint mobility and to withstand mechanical load on the joint (Buckwalter and Mankin, 1998; Bhosale and Richardson, 2008). Its extensive ECM has a unique composition to meet the specific needs of the tissue (Mow et al., 1992). It consists mainly of collagens and heavily charged proteoglycans. A variety of non-collagenous matrix proteins add to the complexity and is important for ECM assembly and tissue maintenance (Heinegård, 2009; Schaefer and Schaefer, 2010).

The articular cartilage matrix is organized differently depending on the distance from the chondrocyte but also depending on the depth from the articular surface (Buckwalter and Mankin, 1998; Guilak et al., 2006; Bhosale and Richardson, 2008; Mittelstaedt et al., 2011). Four zones are distinguished morphologically from the articular cartilage surface to the cartilage–bone interface (Kato et al., 1988; Buckwalter and Mankin, 1998; Hunziker et al., 2002; Bhosale and Richardson, 2008; Fig. 1). The thin superficial zone is characterized by flattened ellipsoid cells and thin collagen fibrils arranged parallel to the surface, providing shear and tensile strength. The intermediate zone contains spheroid-shaped cells and randomly arranged fibres. The deep zone is rich in glycosaminoglycans and shows spheroid-shaped chondrocytes, which are arranged in columns perpendicular to the surface (Youn et al., 2006). The zone of calcified cartilage connects to the subchondral bone (Redler et al., 1975; Hoemann et al., 2012).

Abbreviations: ECM, extracellular matrix; iTRAQ, isobaric tags for relative and absolute quantification; LTP, lateral tibial plateau; MS, mass spectrometry; MTP, medial tibial plateau; MRM, multiple reaction monitoring.

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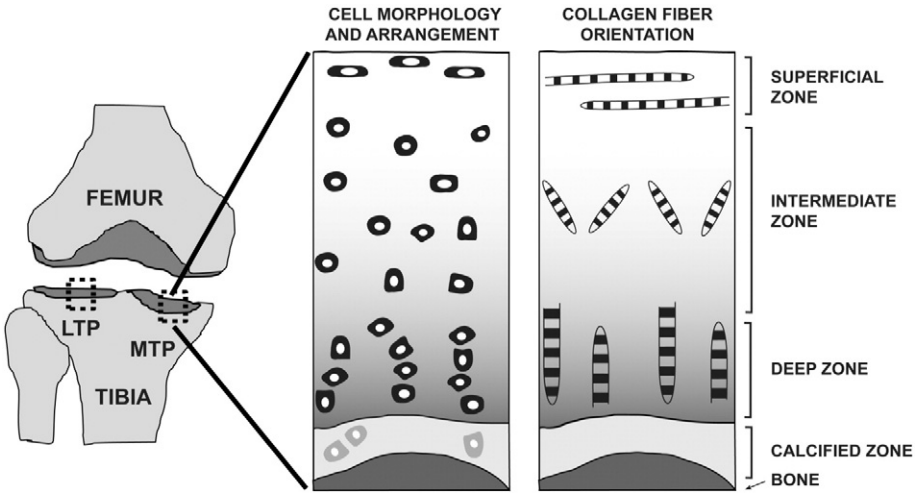

Fig. 1. Articular cartilage zones. Four zones can be distinguished morphologically in articular cartilage. The boxes represent the sample plugs taken from the lateral tibial plateau (LTP) and the medial tibial plateau (MTP). The insert illustrates the cell distribution and the collagen fibril orientation across the full thickness cartilage.

Events that lead to changes in this intricate organization may lead to the development of pathological conditions (Heinegård, 2009). Mechanical injury or disturbances of tissue maintenance, as for example in osteoarthritis, may trigger cells to alter the ECM structure and thus impair the mechanical strength of the tissue (Hunziker, 2002). To understand pathological processes and allow more accurate diagnosis and tailor-made therapy of joint diseases, it is important to know the molecular composition of the tissues involved, including variations between the different zones of the cartilage.

In the past, several studies have been undertaken to characterize these zones with regard to structural organization (Hunziker et al., 2002; Schumacher et al., 2002; Youn et al., 2006), and protein localisation (Schumacher et al., 1994; DiCesare et al., 1995; Lorenzo et al., 1998a; Pfister et al., 2001; Söder et al., 2002; Veje et al., 2003). Generally, these studies focused on one or two components, due to technical restrictions, and could therefore not provide a broad overview. Recently, genomic (Fukui et al., 2008; Grogan et al., 2013) and proteomic studies (Zhang et al., 2011; Cillero-Pastor et al., 2013) were applied to investigate gene expression and protein distribution patterns with a broader scope. Challenges with articular cartilage proteomics include the scarce availability of healthy human articular cartilage biopsies, the high degree of cross-linking between the ECM molecules, and the difficulty in identifying low abundant proteins, particularly in the presence of highly abundant proteins and proteoglycans such as collagens and aggrecan.

The aim of the present study was to quantitatively analyze extractable proteins in human articular cartilage to provide a detailed overview of the protein distribution. This was achieved by first applying a non-targeted mass spectrometry approach (iTRAQ technology) and subsequently by analyzing protein distribution in detail using a targeted approach (MRM technology; Fig. 2).

We found unique patterns of protein distribution throughout the different zones of the articular cartilage. This detailed characterization strengthens our understanding of articular cartilage matrix composition and forms the basis for further investigations of key molecules in tissue function, maintenance and pathology.

2. Results

2.1. Quantitative mass spectrometry—discovery approach

To test the feasibility of the study and to get an overview about the extractable ECM proteins in articular cartilage, iTRAQ labeled protein mixtures of the different depth zones of a LTP sample were analyzed using Q-TOF tandem mass spectrometry. This discovery approach allowed the quantification of 277 proteins. The total protein list, including the weighted average protein ratio (versus reference sample) for each sample pool (pools 1–9) is presented in Supplementary Table S1. Each iTRAQ set was run individually and analyzed separately by calculating the sample-to-reference ratio. The number of peptide ratio measurements for each identified protein varied from $n = 1$ (in a few instances listed in italics in Table 1 and Supplementary Table S1) to approximately 100 for, e.g. collagen and fibronectin. To focus the analysis on cartilage ECM proteins, a subset of 70 proteins was selected (Table 1). Plasma proteins, membrane proteins and intracellular proteins were excluded (Supplementary Table S2). Additionally, collagens except collagen type VI were removed from the list. Due to the high degree of cross-linking in collagens, except collagen type VI, these molecules are poorly extracted and cannot be quantitatively analyzed. Different distribution patterns of ECM proteins in the LTP sample were found (Fig. 3). Several proteins showed a distinct preference for the superficial region, e.g. asporin, tenascin-C and collagen type VI (Fig. 3a). Others were found to be more abundant with increasing depth, e.g. aggrecan (G1, G2 and G3 domains), hyaluronan and proteoglycan link protein 1 (link protein) and chondroadherin (Fig. 3c). Some showed an even distribution throughout the tissue, e.g. decorin and PRELP (Fig. 3d). Only thrombospondin-1 and mimecan were found to be enriched in the middle region (Fig. 3b).

2.2. Data verification using MRM mass spectrometry

From the 70 cartilage ECM proteins identified by the iTRAQ approach, 29 proteins of special interest in articular cartilage were selected for analysis by the MRM technology (marked in Table 1). The advantage of the MRM approach is that pre-optimized assays for selected, unique peptides are used, which provides higher sensitivity. Furthermore, we were able to measure the proteins using a single chromatography step without prior ion-exchange chromatography enabling us to instead take more data points across the cartilage depth, thereby achieving enhanced detail in the protein distribution patterns.

Quantitative analysis of the protein distribution in the LTP sample via iTRAQ and MRM approaches resulted in similar protein distribution patterns for all analyzed proteins (Fig. 3). After having established the feasibility of the study, the MRM approach was now used for a detailed analysis of protein distribution patterns in four MTP samples. The MRM transition list and the quantitative data for each peptide, allowing the quantification of the respective protein in the LTP and the four MTP samples, are shown in Supplementary Table S3 and S4a-e.