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Regional differences of tibial and femoral cartilage in the chondrocyte gene expression, immunhistochemistry and composite in different stages of osteoarthritis

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ABSTRACT

The function of articular cartilage as an avascular tissue is mainly served by collagen type II and proteoglycan molecules. Within this matrix homeostasis between production and breakdown of the matrix is exceptionally sensitive.

The current study was conducted to identify regional differences in specific alterations in cartilage composition during the osteoarthritic process of the human knee joint. Therefor the changes in the expression of the key molecules of the extracellular matrix were measured in dependence of the anatomical side (femoral vs tibial) and associated with immunohistochemistry and quantitative measurement.

60 serial osteochondral femoral condyle and the tibial plateau samples of patients undergoing implantation of total knee endoprosthesis of areas showing mild (Group A, macroscopically ICRS grade 1b) respectively advanced (Group B, macroscopically ICRS grade 3a/3b) (30 each) osteoarthritis according to the histological-histochemical grading system (HHGS) were compared with 20 healthy biopsies with immunohistochemistry and histology. We quantified our results on the gene expression of collagen type I and II and aggrecan with the help of real-time (RT)-PCR. Proteoglycan content was measured colorometrically.

In group A slightly increased colour intensity was found for collagen II in deeper layers, suggesting a persisting but initially still intact repair process. But especially on the medial tibia plateau the initial Col II increase in gene expression is followed by a decrease leading to the lowest over all Col II expression on the medial plateau, here especially in the central part. There in late stage diseases the collagen type I expression was also more pronounced. Markedly decreased safranin O staining intensity was observed in the radial zone and less reduced intensity in the transitional zone with loss of zonal anatomy in 40% of the specimens in group A and all specimens in group B. Correlation between colorometrically analysed proteoglycan GAG content and aggrecan Real Time PCR is mainly weak.

Tibial and femoral cartilage in contrast to patellar cartilage both are preferential exposed to compressive stresses, but presence of menisci affects the load distribution at the tibial side, which creates varying conditions for the different cartilage surfaces in the knee.

As directly measured Poissonís ratio in tibial cartilage is higher but Youngís modulus is lower than in femoral cartilage, different resulting feedback amplification loops interact with proceeding cartilage damage. The initial loss of aggrecan may support Matrix metalloproteinases (Mmps) in the access to the collagen network and the considerably differing mechanical properties at both joint surfaces result in varying increased synthesis and release of matrix degrading enzymes.

The present study has identified a selection of events which reflect the response of cartilage structure and composite, chondrocytes itself and their productivity to changes in mechanical stress depending on the anatomical site.

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1. Introduction

The survival and integrity of articular cartilage and especially its extracellular matrix depends mainly on a balance between synthesis and depletion of matrix molecules which largely serve its primary function to transmit mechanical loads.

Concerning clinical studies little is known about the early development that takes place in hyaline cartilage due to the asymptomatic nature of the early disease.

Mainly animal studies revealed existing differences between cartilage regions of one individual, for example demonstrated by regional differences in RNA levels in areas of the rabbit knee joint. In the human so far area specific alterations in cartilage composition have been shown by altering contents and compositions of glycosaminoglycans at different sites of the human hip joint (Yoshida and Azuma 1982). Chondrocytes in the different vertical cartilage zones demonstrate significant variation in characteristics, such as matrix synthesis activity (Martin et al., 2001; Lorenz et al., 2005) and amount of those differences is dependent on the anatomical site as well as metabolic variations (Atayde et al., 2012), too. In many studies semi- quantitative reverse transcriptase- polymerase chain reaction (RT-PCR) has been proven to reveal reliable and reproducible assessment of cell producibility. The advantages and limitations of this method have been discussed extensively as other authors such as Mahjoub et al. (2012) have developed different kinds of comparative or absolute quantification. For instance methods indicating a specific number of Col I mRNA molecules per cell or weight of cartilage must be assessed very critically as several problems exist, e.g. the necessity of a plasmid standard for every gene or the high number of work steps associated with inaccuracies of unknown or hardly measurable amount. Besides rewriting of mRNA into cDNA is not efficient for every gene as several sequence dependent differences exist. In so far after the first description of RT-PCR as a method to allow quantification of levels of ECM (extracellular matrix) gene expression among all cartilage layers the method has been widely accepted as being more sensitive compared to other techniques, for example because it is based on Ct values established in the early exponential phase of the PCR reaction, when none of the reagents is rate limiting, standing in contrast to end point measurement of the amount of accumulated products.

Overmore in case of the knee joint very few studies deal with differences between tibial and femoral surfaces mainly analysing radiological aspects (Everhart et al., 2014), although mechanical properties differ considerably at both joint surfaces (Chokhandre et al., 2015) resulting in an increased synthesis and release of matrix degrading enzymes. The natural effect of this release is the disturbance of the pericellular matrix increasing the chondrocyteís exposure to collagen type II fibrils naturally only found within territorial and interterritorial site of healthy articular cartilage (Polur et al., 2010). In many cases already radiological comparisons rsp follow-ups show significant differences in the appearance of the affected joint surfaces and subchondral bone structures, for example concerning subchondral sclerosis.

So far regional differences in knee cartilage properties were limited to estimations about the tibiofemoral joint space to reason cartilage thickness. Meanwhile non-invasive cartilage thickness measurement mainly is provided to construct a three- dimensional thickness map by segmenting consecutive MR images creating mean thickness measurements on the map. However although those measurements have revealed significant findings about the development of knee osteoarthritis mainly they are still limited to advanced OA changes and do not deliver qualitative information. More recent studies such as Favre et al. (2013) using bi-orthogonal thickness patterns that were extracted from thickness maps of segmented magnetic resonance images in the medial, lateral and trochlea compartments showed that thickness patterns are relatively similar among asymptomatic knees but show differences with increasing OA severity and are more sensitive than mean thickness measurements. Nevertheless little is known about the regional quantification of hyaline cartilage composition during the OA process concerning especially the matrix synthesis activity. In the present study the changes in the expression of the key molecules of the extracellular matrix were measured in dependence of the anatomical side (femoral vs. tibial, medial and lateral each) and associated with immunohistochemistry and cartilage composition to identify regional differences during the osteoarthritic process.

2. Methods

2.1. Patients and samples

Osteochondral samples (4 samples per patient) with lesions macroscopically and intraoperatively graded as ICRS grade 1b and with ICRS grade 3a or 3b lesions were taken from the lateral and medial femoral condyles and the lateral and medial tibial plateaus from a continuous series of 95 patients undergoing implantation of total knee endoprosthesis following ICRS guidelines for standardized histoprocessing and unbiased evaluation of human biopsies (Hoemann et al., 2011). The samples were drawn at a right angle from as deep as the subchondral bone taking care not to sample tissue from the joint margins or osteophyte and were presented blinded with respect to tissue origin and location and examined twice in random order under direct light microscopy by 2 independent observers.

To standardize the following examinations in these preliminary groups we identified 30 samples of each of these 4 areas with a Collins and McElligott histopathological grading (Miosge et al., 2004) of I and III and with a comparable grading (mild vs. severe OA changes with a grading of 4 or 5 points vs. 10–12 points respectively) in the “histological-histochemical grading system” (HHGS) for osteoarthritis scores, the modified “Mankin Score” (Ehrlich et al., 1986).

Those two groups were defined as group A (mild OA with recognizable cracks and fissures) and group B (severe OA) respectively.

The 30 patients with samples in group A ranged in age from 56 to 73 years (average: 64 years, 18 women, 12 men), those 30 in group B from 56 to 74 years (average: 65 years; 19 women, 11 men). Twenty control biopsies with macroscopically healthy cartilage (ICRS grade 0) were taken from the same region, 12 of these taken during an ACI (autologous chondrocyte implantation), 7 during endoprosthesis implantation (mainly after extra-articular resection of the distal femur and the proximal tibia and reconstruction with a tumour endoprosthesis) and 1 during major amputation (age: 26–68 years, average: 47 years; 10 women and 10 men). We excluded patients with rheumatoid arthritis, ankylosing spondylitis, psoriasis or similar arthritides, and malignancies or infections with inflammatory arthritides. The control group included samples with a Collins and McElligott histopathological grading of 0 and normal articular cartilage in the “histological-histochemical grading system” (HHGS) for osteoarthritis (0 or 1 point).

Approval was given by the institutional review board, and informed consent was obtained from each patient.

2.2. Fixation and staining

After tissue prefixation and decalcification in EDTA the sections were dehydrated, and Epon (1:1) embedded (Epon 812, Fa. Carl Roth GmbH, Karlsruhe). Formalin fixation, slicing into semithin sections (5 μm), and paraffin staining were performed according to standardized procedures. All histological sections (x50