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RESEARCH ARTICLE

Mapping the secretome of human chondrogenic progenitor cells with mass spectrometry

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ABSTRACT

Tissue engineering offers promising perspectives in the therapy of osteoarthritis. In the context of cell-based therapy, chondrogenic progenitor cells (CPCs) may be used to regenerate defects in cartilage tissue. An in-depth characterization of the secretome of CPCs is a prerequisite to this approach. In this study, a method was developed for the qualitative and quantitative analysis of the secretome of undifferentiated and differentiated CPCs. Secreted proteins from cells grown in two-dimensional as well as three-dimensional alginate cultures were extracted and analyzed by liquid chromatography/tandem mass spectrometry (LC–MS/MS). Quantitation was achieved by internal standardization using stable isotope-labeled amino acids in cell culture (SILAC). Qualitative analysis of CPC secretomes revealed ECM-components, signal proteins and growth factors most of which were also found in healthy cartilage. A quantitative comparison revealed significantly upregulated proteins with regenerative potential during differentiation, while proteins involved in catabolic metabolism were significantly downregulated. The development of methods for qualitative and quantitative analysis of the secretome of CPCs by mass spectrometry provides a foundation for the investigation of progenitor or stem cells from other sources.

1. Introduction

Osteoarthritis (OA) is one of the most frequent diseases. It has great socio-economic impact and often leads to severe pain (Luong et al., 2012). Therapeutic interventions lead to an alleviation of symptoms; however, the injured cartilage cannot be regenerated (Lohmander and Roos, 2007). Healthy cartilage mainly consists of an extensive extracellular matrix (ECM) in which numerous proteins organize the metabolism of the chondrocyte. Disturbed cell-matrix interactions play a central role in the pathogenesis of OA. These defects are characterized by an imbalance between catabolic and anabolic processes of chondrocytes which gradually lead to a restructuring of the ECM (Loeser, 2006; Goldring and Goldring, 2007; Heinegard and Saxne, 2011). The histological analysis of late-stage osteoarthritis typically shows deep fissures, multiplications of the tidemark, and chondrocyte clusters (Fig. 1).

At this stage, breaks in the subchondral bone occur, resulting in the opening of marrow spaces. The mineralized zone of cartilage tissue vascularizes. CPCs migrate into the damaged cartilage and begin to synthesize fibrocartilaginous repair tissue. This tissue does not contain collagen type II, and cannot permanently withstand forces in the joint (Cingoez, 2015). These CPCs exhibit stem cell characteristics such as clonogenicity, multipotency, migratory activity and especially a high chondrogenic potential (Koelling et al., 2009). Promising discoveries in the field of tissue engineering suggest that CPCs might be used in the future to regenerate cartilage destroyed by osteoarthritis (Kruegel et al., 2008). To achieve this aim it is necessary to manipulate CPCs so that they synthesize an ECM with an intact fiber network containing collagen type II. Koelling et al. cultivated CPCs in three-dimensional alginate culture and showed that

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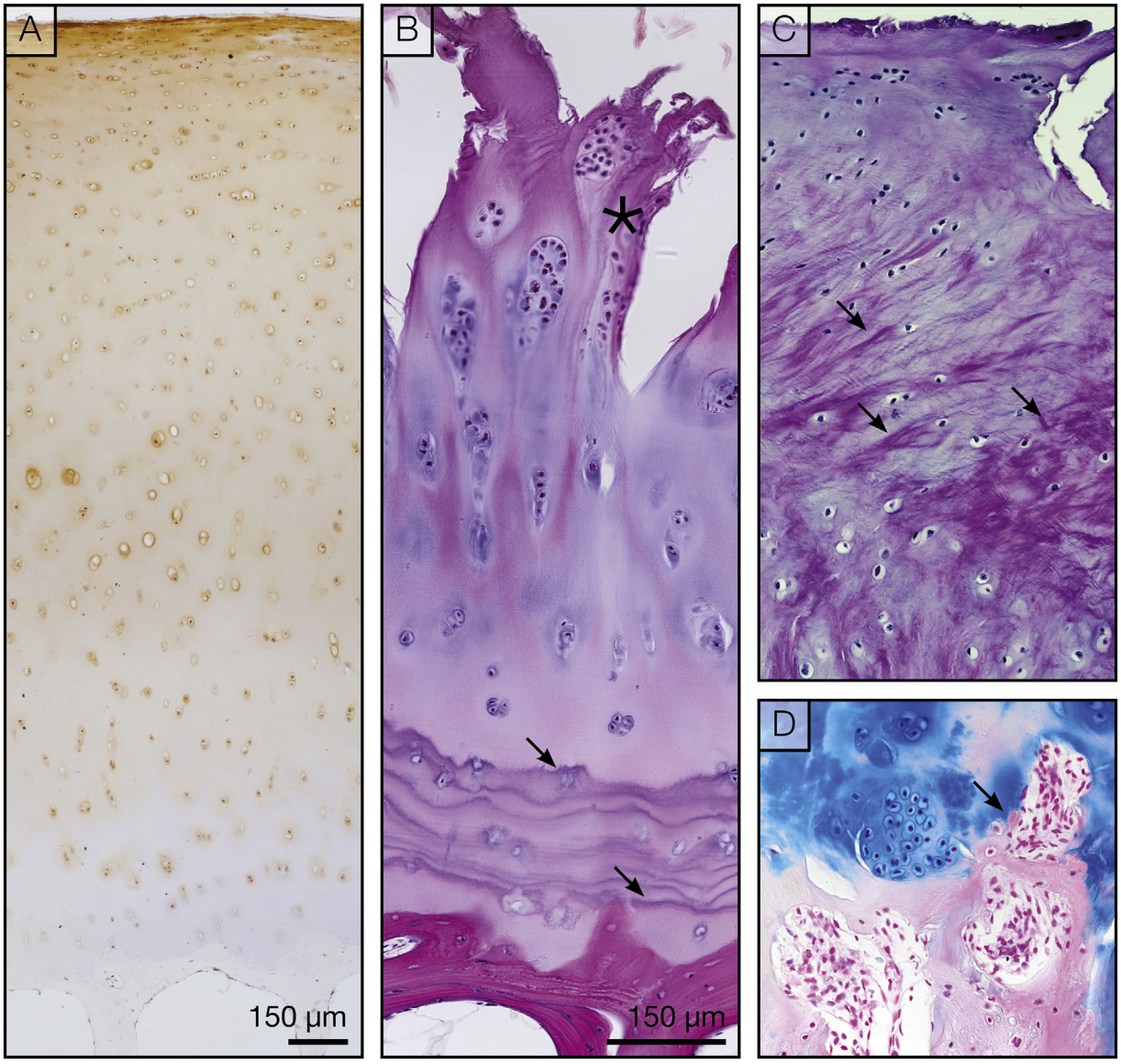

Fig. 1. Histological section of the articular cartilage (vertical section/knee). A: Healthy articular cartilage (Laminin staining β1) B: late-stage OA. Typical signs: deep fissures; chondrocyte clusters (*); multiplication of the idemark (arrows). C: Hyaline cartilage traversed by fibrocartilagenous repair tissue. D: Bone marrow cells (arrow) migrate into the mineralized zone of the articular cartilage (B–D HE-staining). Figure kindly provided by Cingoez (2015).

they are indeed capable of synthesizing an ECM containing collagen type II ex vivo (Kruegel et al., 2008; Koelling et al., 2009).

The aim of our investigation has been to determine the secretome of CPCs; i.e. the entire repertoire of proteins secreted into the extracellular space, in order to obtain a better understanding of CPC metabolism. In particular, we intended to clarify the question of whether the differentiation of these cells (by cultivation in three-dimensional medium) enhances their chondrogenic potential, and to identify the proteins mediating this change. To answer these questions, we cultivated CPCs in 2D and 3D cultures for proteomic analysis by liquid chromatography/tandem mass spectrometry (LC-MS/MS). In addition, we used SILAC (Stable Isotopic Labeling of Amino acids in Cell Culture) standardization to enable a quantitative comparison between undifferentiated CPCs in monolayer (2D CPCs) and CPCs cultured in alginate (3D CPCs). We used CPCs from patients undergoing total knee replacement as a model system. If it is possible to manipulate these cells to secrete an intact repair tissue, it should be possible to transfer our findings to progenitor cells from other joints, e.g. the temporomandibular joint (Robinson et al., 2015).

2. Material and methods

2.1. Tissue collection, cell isolation, immortalization and cell culture

Fibrocartilage samples were collected during knee replacements from adult patients suffering from late stage gonarthrosis, with permission of the Ethics Committee of the University of Göttingen (No. 25/12/10). Informed consent was obtained for experimentation with human subjects. A rheumatic disease was histologically excluded. The disease was defined by the criteria of the "American College of Rheumatology Classification Criteria" (Altmann et al., 1986). To facilitate cell migration, the fibrocartilage samples were scraped with a scalpel to gain small pieces of tissue explants. The explants were placed in 75 cm^2 culture flasks and were raised with Dulbecco's modified Eagle's medium (DMEM) + GlutaMax™, supplemented with 10% fetal bovine serum (GIBCO, lot number 41F2061K) and 50 $\mu g\,ml^{-1}$ gentamycin (PromoCell) under standard conditions (37 °C, 5% CO_2, 95% humidity). The explants were placed for 10 days and not moved or shaken dur-