Migratory chondrogenic progenitor cells from late stages of osteoarthritis exhibit gender differences

Sebastian Koelling[1], Jenny Kruegel[1], Malte Irmer[1], Jan Ragnar Path[1], Boguslawa Sadowski[1], Xavier Miro[2], Christa Bode[1], Nicolai Miosge[1]

[1]Tissue Regeneration Group, Medical Faculty, Department of Prosthodontics, Georg August University, [2]Department of Molecular Cell Biology, Max-Planck-Institute for Biophysical Chemistry, Goettingen, Germany

INTRODUCTION: The regeneration of diseased hyaline cartilage continues to be a great challenge, mainly because degeneration—caused either by major injury or by age-related processes—can overextend the tissue's self-renewal capacity.

METHODS: Tissue sources and preparation Adult osteoarthritic cartilage, without signs of rheumatoid involvement, was obtained from the knee joints of patients (ages: 65 – 75 years) suffering from late-stage OA after total knee replacement. For light microscopy, tissue samples were processed for immunohisto-chemistry as described elsewhere (Bock et al. 2001). For ultrastructural investigations, 8 mm^3 cartilage samples from the upper intermediate zones were resected from areas adjacent to the main defect at a maximum distance of 0.5 cm. **Cell isolation and culture** Standard explant cultures were performed using 8-15 mm^3 tissue specimens taken from areas adjacent to the main defect. After 10 days, outgrown cells were harvested and 10^3 cells/cm^2 were transferred to a monolayer culture in Dulbecco´s modified Eagle´s medium (DMEM) with 10% fetal bovine serum (GIBCO, Lot. nr. 41F2061K), supplemented with penicillin/streptomycin (50000 U/50mg) and L-Glutamine (10 mM) and cultured under standard conditions. Population doublings were calculated as described elsewhere (Bi et al, 2007). **Cloning and immortalization** We performed dilution cloning with 10 cells/ml and 100 µl/well on a microtitration plate with 3 individual CPC populations. Lentivirus expressing hTERT was produced as described elsewhere (Docheva et al. 2009). **Multipotent differentiation** A number of 10^3 CPC/cm^2 samples in 75 cm^2 flasks were encouraged toward the osteogenic lineage by applying NH OsteoDiffMedium™ (Miltenyi Biotec) and For adipogenesis, we used NH AdipoDiffMedium™. For chondrogenic differentiation, a 3D-alginate culture was initiated when 70% confluence in the monolayer was reached. Light microscopy immunofluorescence Primary cells were transferred to P1 in 96-well plates. After 16 h, they were fixed with 70% ethanol and incubated with 100 µl of primary antibody diluted 1:50 in PBS for 1 h at RT in the dark. When necessary, we followed this procedure with a secondary fluorescence-coupled antibody diluted 1:500 for 20 min at RT. Two washing steps with PBS and DAPI staining were performed thereafter.

Quantitative real-time RT-PCR Quantities of 5 µl of RealMasterMix (2.5x)™ (Eppendorf, Hamburg, Germany), 20 pmol of each primer and 1 ng of cDNA were added to a final volume of 10 µl. Primers were designed with the help of primer3® (http://frodo.wi.mit.edu/cgibin/primer3/primer3) and can be found at http://www.prothetik.med.uni-goettingen.de/nmiosge.

RESULTS: Recently, we showed, that repair tissue from late stages of osteoarthritis in humans harbors a unique progenitor cell population, termed chondrogenic progenitor cells (CPCs). These exhibit stem cell characteristics such as clonogenicity, multipotency, and migratory activity. The isolated CPCs were shown to populate diseased tissue ex vivo. Down-regulation of the osteogenic transcription factor runx-2 enhanced the expression of the chondrogenic transcription factor sox-9. This, in turn, increased the matrix synthesis potential of the CPCs. Unpublished results show that gender differences of the CPCs exist and that they exhibit estrogen and progesterone receptors. Treatment, especially with estrogen, at least in vitro, can enhance their chondrogenic potential.

DISCUSSION & CONCLUSIONS: Our results offer new insights into the biology of progenitor cells in the context of diseased cartilage tissue. Our work may be relevant in the development of novel therapeutics for the later stages of osteoarthritis.