CEP-48: A NEW MARKER GENE PREFERENTIALLY EXPRESSED IN CULTURED CHONDROCYTES

*Steck, E; *Lorenz, H; **Gress, T; *Loew, M; +*Richter, W

+*Department of Orthopaedic Surgery, Heidelberg, Germany. PD Dr. Wiltrud Richter, Stiftung Orthopädische Universitätsklinik Heidelberg, Schlierbacher Landstr. 200a, 69118 Heidelberg, Germany, +49 6221 969254, Fax: +49 6221 969288, wiltrud.richter@ok.uni-heidelberg.de

Introduction: Cell based therapies using ex vivo cultured autologous chondrocytes are of potential use to augment the limited healing capacity of cartilage defects and prevent osteoarthritis. This kind of therapy however requires highly invasive procedures and generation of new cartilage defects to harvest the autologous chondrocytes from the patient. Therefore, mesenchymal stem cells derived from bone marrow may be a superior source for autologous cells of chondrogenic potential. To identify chondrocyte-like cells in tissue culture and distinguish them from mesenchymal stem cells or osteoblast-like cells, chondrocyte-specific marker genes are of great interest. We here searched for genes expressed in cultured chondrocyte derived from articular cartilage which are not expressed in mesenchymal stem cells or osteoblast-like cells cultured from bone.

Methods: Primary cell cultures of human cartilage and bone were obtained from the humeral head after accidental fracture and grown in DMEM with 10% FCS. Mesenchymal stem cells were isolated from bone marrow by density gradient centrifugation and cultured under the same conditions. RNA was isolated from chondrocyte monolayers at passage 2 and from osteoblast monolayers at passage 1, after culturing the cells for 6 and 8 weeks, respectively. The mRNA was reverse transcribed to cDNA and subtractive gene expression analysis was performed by representational difference analysis (RDA). A tester to driver ratio of 1:100 was used to generate the first differential product (DP1). 1:800 and 1:400,000 ratios were applied in the two following subtraction rounds yielding DP2 and DP3. Finally DP3 PCR-bands were isolated and subcloned into plasmid vectors for sequencing. Differentially expressed genes were analyzed by Northern blot hybridization and by semi-quantitative RT-PCR with gene specific primers using collagen Type 2 (Col2) as a cartilage specific marker gene and GAPDH as a control.

Results: Eight distinct chondrocyte-specific gene fragments were identified in DP3 (Fig.1). Six fragments belonged to the gene for the cartilage glycoprotein 39 (GP-39, YKL-40) and one to the human YKL-39 precursor protein, confirming the preferential expression of these genes in cultured chondrocytes. The last fragment was highly homologous to a human cDNA-sequence with unknown function and to a much shorter rat sequence. By Northern Blot analysis we identified a transcript of 3.1 kb length, specifically expressed in cultured chondrocytes but not in osteoblast-like cells (Fig.2). Sequencing of the corresponding full length cDNA amplified from human chondrocytes revealed a new ORF coding for a 451 amino acid protein harboring one EGF-like, calcium-binding domain. An insertion of 41 bp within the ORF leading to a reading frame shift distinguishes this new protein from the homologous database sequence, where no EGF-like domain is present. The expected protein with a calculated molecular weight of approximately 48 kDa was named chondrocyte expressed protein (CEP-48). Semi-quantitative RT-PCR experiments on RNA isolated from at least two different donors revealed CEP-48 gene expression in cultured chondrocytes but not in cultured osteoblast-like cells grown from bone explants and in mesenchymal stem cells. Cartilage and primary bone tissue however were both positive for CEP-48 (Fig.3).

Discussion: Using cDNA-RDA we identified a new member of the EGF-like gene family: CEP-48. Beside the EGF-like calcium-binding domain with 6 conserved cystein residues CEP-48 shows no significant sequence homology to any other domain of known extracellular matrix or growth inducing proteins. Since this gene is expressed in cultured chondrocytes as well as in cartilage tissue but not in mesenchymal stem cells and cultured osteoblasts, it may serve as a new marker gene for stem cell based chondrogenic tissue engineering.

Discussion: Using cDNA-RDA we identified a new member of the EGF-like gene family: CEP-48. Beside the EGF-like calcium-binding domain with 6 conserved cystein residues CEP-48 shows no significant sequence homology to any other domain of known extracellular matrix or growth inducing proteins. Since this gene is expressed in cultured chondrocytes as well as in cartilage tissue but not in mesenchymal stem cells and cultured osteoblasts, it may serve as a new marker gene for stem cell based chondrogenic tissue engineering.