Chondrogenic Differentiation Capacity of Human Mesenchymal Progenitor Cells Derived from Subchondral Cortico-Spongious Bone

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ABSTRACT: Microfracture is frequently used to repair articular cartilage defects and allows mesenchymal progenitors to migrate from subchondral bone into the defect and form cartilaginous repair tissue. The aim of our study was to analyze the cell surface antigen pattern and the differentiation capacity of cells derived from human subchondral bone. Human progenitor cells were derived from subchondral cortico-spongious bone and grown in the presence of human serum. Stem cell-related cell surface antigens were analyzed by flowcytometry. Cortico-spongious progenitor (CSP) cells showed presence of CD73, CD90, CD105, and STRO-1. Multilineage differentiation potential of CSP cells was documented by histological staining and by gene expression analysis of osteogenic, adipogenic, and chondrogenic marker genes. CSP cells formed a mineralized matrix as demonstrated by von Kossa staining and showed induction of *osteocalcin*, independent of osteogenic stimulation. During adipogenic differentiation, the adipogenic marker genes *fatty acid binding protein 4* and *peroxisome proliferative activated receptor* γ were induced. Immunohistochemical staining of cartilage-specific type II collagen and induction of the chondrocytic marker genes *cartilage oligomeric matrix protein*, *aggrecan,* and *types II* and *IX collagen* confirmed TGFβ3-mediated chondrogenic lineage development. CSP cells from subchondral bone, as known from microfracture, are multipotent stem cell-like mesenchymal progenitors with a high chondrogenic differentiation potential. © 2008 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 26:1449–1456, 2008

Keywords: microfracture; stem cells; cartilage repair; chondrogenesis

Injuries of the articular cartilage of the knee are common and were documented in about 60% of the patients subjected to knee arthroscopies.[1,2] These cartilage lesions are predominantly classified as focal chondral or osteochondral lesions (67%) and osteoarthritic changes (29%). The majority of defects (70%) are nonisolated cartilage lesions.[3] Since injured articular cartilage does not heal and has only a low regenerative capacity, reparative surgical therapy options aim at the reconstruction of the articular surface and formation of adequate cartilage repair tissue. In particular, techniques that stimulate the bone marrow like Pridie-drilling, abrasion, or the microfracture technique are frequently used for the treatment of focal cartilage defects.[4–6] These techniques have in common that mesenchymal progenitor cells from the subchondral spongious bone marrow are allowed to populate the defect and subsequently form a cartilaginous repair tissue. For instance, the minimally invasive microfracture technique induces the healing sequence by directly accessing the subchondral bone marrow within the defect site. Arthroscopically, the cartilage lesion is subjected to debridement, while damage to the subchondral bone plate is avoided and a firmly attached healthy cartilage rim is prepared. Access to the bone marrow is established by introducing multiple, evenly distributed perforations across the defect and adjacent to the cartilage rim. Bleeding is observed and blood-derived cells and bone marrow-derived mesenchymal stem and progenitor cells are flushed into the defect, forming a blood clot. It is suggested that growth factors from the subchondral bone or even from the synovial fluid may stimulate these progenitors to form a cartilaginous repair tissue of a nonhyaline appearance, filling the defect.[7–9] Clinically, the outcome after microfracture treatment of full-thickness cartilage defects showed good and satisfactory short-term results in up to 77% of patients.[10–12] Patients showed significant clinical improvement, 2 and 5 years after microfracture, compared to the preoperative situation as assessed by the Lysholm, Tegner and SF-36 physical component score.[10,11] However, in a group of 85 patients with full-thickness cartilage defects, it has been shown that microfracture treatment significantly improved the clinical situation as assessed by the International Cartilage Repair Society (ICRS) score at 18 months. In the long-term, at 36 months, the ICRS score was significantly higher compared to the preoperative situation, but was significantly decreased compared to the 18 months follow-up scores.[12] Therefore, microfracture treatment shows good short-term results, but clinical results may become impaired in the long-term.

From the cellular point of view, mesenchymal progenitor or stem cells from bone marrow, e.g. isolated from iliac crest, have a multipotential differentiation capacity that allows development along the chondrogenic, osteogenic, and adipogenic lineage.[13] These cells can be isolated, extensively grown in vitro while maintaining

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their undifferentiated, multipotent status, and may gradually loose their proliferation and differentiation capacity with an increasing number of cell passages.[14] Taking the differentiation capacity and plasticity of MSC, as well as their low inherent immunogenicity into account, in recent years, much attention has been drawn to stem cells as promising candidates for cell-based approaches in regenerative medicine. For instance, clinical studies and case reports have been reported using in vitro propagated bone marrow-derived stem cells from iliac crest for the restoration of articular cartilage surfaces in osteoarthritic lesions and for filling of focal articular defects in combination with a collagen gel. Clinically, symptoms improved significantly and the defects were filled with hyaline-like tissue or fibrocartilage, histologically.[15–17] However, the repair tissue induced by microfracture and formed by subchondral mesenchymal progenitors has been shown to be of a cartilaginous to fibrous appearance with limited short-term durability. Therefore, improvement of the microfracture technique is indicated that may enhance cartilage matrix deposition and improve the cartilaginous repair tissue.[18] The knowledge about human mesenchymal progenitor cells derived from the subchondral bone marrow as known from microfracture and their multipotential differentiation capacity is limited. Therefore, the aim of our study was to comprehensively characterize the differentiation capacity and the phenotype of these progenitors that may contribute to the formation of cartilage repair tissue after microfracture. Emphasis was placed on the chondrogenic lineage development of human subchondral cortico-spongious progenitors. We hypothesize that these cells derived from the cortico-spongious subchondral bone marrow have a multipotential differentiation capacity and may present typical cell surface antigens known from human mesenchymal stem cells derived from bone marrow aspirates.

METHODS

Isolation and Cultivation of Human Cortico-Spongious Progenitor Cells

Human subchondral cortico-spongious bone chips were obtained from six donors (four females, two males; age 39–62 years) from the lateral tibia head during high tibial closed wedge osteotomy as described previously.[19] The study was approved by the ethical committee of the Charité–Universitätsmedizin Berlin. To harvest human cortico-spongious progenitor (CSP) cells, bone chips were cut into small fragments, washed with phosphate buffered saline (PBS, Biochrom, Berlin, Germany), and partially digested for 4 h at 37°C using 256 U/ml collagenase XI (Sigma, Taufkirchen, Germany). The supernatant was discarded and the remaining fragments were placed in primaria culture flasks (Becton Dickinson, Heidelberg, Germany) and cultured in OptiPro SFM medium (Invitrogen, Carlsbad, CA) containing 5% human serum (German Red Cross, Berlin, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (all Biochrom, Berlin, Germany). Cells that reached 80%–90% confluence were passaged using trypsin-EDTA in PBS (0.05% v/v, Biochrom) and re-plated at a density of 8,000 cells/cm^2. Medium was exchanged every 2–3 days.

Flowcytometry Analysis

CSP cells (2.5×10^5 cells, passage 3) were washed in PBS/0.5% BSA and incubated with monoclonal mouse anti-human antibodies directly conjugated with fluorochromes for 15 min on ice. For indirect staining, cells were incubated 15 min either with biotin-labeled mouse anti-human SH-2 or STRO-1-IgG, washed with PBS/0.5% BSA and stained with streptavidin-allophycocaine (SA-APC) for SH-2 or rabbit-anti-mouse-IgG-fluorescein-isothiocyanate (FITC, Dako, Hamburg, Germany) for STRO-1 for 10 min. Staining of cell surface antigens was analyzed using the FACSCalibur equipped with CELLQUEST software (Becton Dickinson). Apoptotic cells were excluded from analysis using propidium iodide (PI). Unstained cells served as negative, and CD3-stained cells as isotypic control. The following antibodies were used: CD73-Phycoerythrin (PE), CD34 PE, CD166 PE, CD44 FITC, CD90 FITC (Becton Dickinson). CD3 labeled to PE, FITC and APC, CD14 PE, CD45RO FITC, biotin-labeled SH-2 and PI were provided by the German Rheumatism Research Center (DRFZ, Berlin, Germany). SA-APC was purchased from Becton Dickinson. STRO-1-IgG was a kind gift from P. Charbord (Equipe INSERM-ESRI, Tours, France).

Cell Differentiation Studies

Chondrogenic differentiation of CSP cells (passage 3) was performed under serum-free conditions in high-density pellet cultures ($n = 5$ donors, 250,000 cells/pellet) as described previously.[20] Chondrogenesis was induced by adding 10 ng/ml transforming growth factor-β3 (TGFβ3, R&D Systems, Minneapolis, MN). The medium was exchanged every 2–3 days and cells were maintained for up to 28 days. For osteogenic and adipogenic differentiation, CSP cells ($n = 3$ donors) were plated at a density of 10,000 cells/cm^2 onto Thermanox® Plastic Coverslips (Nunc, Wiesbaden, Germany). For osteogenic differentiation, confluent monolayer cultures were stimulated with low-glucose DME-medium containing 5% human serum and osteogenic supplements (0.1 μM dexamethasone, 50 μM L-ascorbic acid-2-phosphate, 10 mM β-glycerophosphate; all Sigma). Cells were cultured for 28 days and medium was changed every other day. For adipogenic differentiation, cells were stimulated 3 days postconfluence with high-glucose DME-medium containing 5% human serum and adipogenic supplements [1 μM dexamethasone, 0.2 mM indomethacin, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; all Sigma), 10 μg/ml insulin (NovoNordisk, Mainz, Germany)]. Cells were cultured for 15 days and controls were maintained without TGFβ3 or adipogenic or osteogenic supplements.

Histological, Histochemical, and Immunohistochemical Staining

Chondrogenic differentiation was assessed by embedding micro-masses in OCT compound, freezing, and cryosectioning (6 μm). Sections were incubated for 30 min with primary rabbit anti-human type II collagen antibodies (Acris, Hiddenhausen, Germany), colorimetrically detected by 3-amino-9-ethylcarbazole (AEC) (EnVision™ + HRP-System, Dako) and counterstained with hematoxylin (Merck, Darmstadt, Germany). Alcian blue 8GS (Roth, Karlsruhe, Germany) staining showed proteoglycans. Osteogenic cells were detected histochemically by analyzing alkaline phosphatase activity using sigma fast BCIP/NBT (Sigma) and by staining of mineralized matrix components according to von Kossa. Intracellular lipid droplets in adipogenic cultures were visualized using Oil Red O (Sigma).

RNA Isolation and Real-Time RT-PCR

Total RNA was isolated from either 25 pellets (chondrogenesis) or cells cultured in 6-well-plates (osteogenesis, adipogenesis) per point in time as described previously.[21] Subsequently, RNA representing chondrogenic ($n = 25$), osteogenic ($n = 2$), adipogenic ($n = 2$) cultures and the respective controls were pooled and 3 μg total RNA was reversely transcribed with the iScript cDNA Synthesis Kit according to the manufacturer's recommendations (BioRad, Munich, Germany). The relative expression level of the housekeeping gene *glyceraldehyde-3-phosphate dehydrogenase* was used to normalize marker gene expression in each sample. Real-time RT-PCR using the i-Cycler PCR System (BioRad) was performed with 1 μl of cDNA sample using the SYBR Green PCR Core Kit (Applied Biosystems, Darmstadt, Germany) in triplicate. Relative quantitation of the marker genes (Table 1) was performed according to the ΔΔ-Ct method and is given as fold change compared to controls.

Statistical Analysis

For statistical analysis of technical reproducibility of gene expression data, the *t*-test was performed. Differences were considered significant when fold change was greater than 2.0 or less than −2.0, and $p < 0.05$.

RESULTS

Morphology and Cell Surface Antigen Pattern of Human CSP Cells

Subchondral cortico-spongious bone fragments were helical in shape, of a yellowish color and 15 × 10 mm in size (Fig. 1A). At 4–10 days after enzymatic digestion, cells started outgrowing from bone fragments (Fig. 1B). Cells firmly attached to the surface of the cell culture plate, presented a spindle-shaped, fibroblast-like morphology, were agranular and formed colonies. Subcultured cells grew in monolayer, maintained a stable fibroblast-like morphology with no signs of granulation (Fig. 1C), and showed a swirl-like growth pattern when reaching confluency (Fig. 1D). Expanded CSP cells showed typical cell surface antigens known from mesenchymal stem and progenitor cells (Fig. 1E). Human CSP cells were a homogenous population and presented the antigens SH-2 (CD105, Endoglin), SH-3 (CD73), THY-1 (CD90), and the activated leukocyte cell adhesion molecule (ALCAM, CD166). CSP cells were homogenously positive (91%–98%) for CD73, CD90, and CD105. The cells showed a variable presentation of CD166, with 30%–85% positive cells. STRO-1 was detected in a minority of cells (4%). Cells were negative for the hematopoietic antigens CD3 (0%) and CD34 (0%), as well as for the leukocyte common antigen CD45 (0.1%) and the lipopolysaccharide receptor CD14 (0.1%).

Chondrogenic Differentiation of Human CSP Cells

To evaluate the chondrogenic differentiation potential of human CSP cells, cells were cultured for up to 28 days in high-density micro-mass cultures under standard chondrogenic conditions and stimulated with TGFβ3. Histological analysis of micro-masses (Fig. 2) showed that CSP cells developed a dense tissue, rich in viable

Table 1. Chondrogenic, Adipogenic, and Osteogenic Marker Genes

Gene Name	Gene Symbol	Accession No.	Oligonucleotides (5′ → 3′) (Up/Down)	Product Size (bp*)	Annealing Temperature (°C)
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	NM_002046	GGC GAT GCT GGC GCT GAG TAC TGG TCC ACA CCC ATG ACG A	149	62
Aggrecan	AGC1	NM_013227	CCA GTG CAC AGA GGG GTT TG TCC GAG GGT GCC GTG AG	146	60
Cartilage link protein	CRTL	NM_001884	GCG TCC GCT ACC CCA TCT CTA GCG CTC TAA GGG CAC ATT CAG TT	145	56.6
Cartilage oligomeric matrix protein	COMP	NM_000095	GGG TGG CCG CCT GGG GGT CTT CTT GCC GCA GCT GAT GGG TCT C	116	59.1
Fatty acid binding protein 4	FABP4	NM_001442	CCT TAG ATG GGG GTG TCC TGG TA AAT GTC CCT TGG CTT ATG CTC TC	156	62
Osteocalcin	BGLAP	NM_199173	GAG CCC CAG TTC CCC TAC CC GCC TCC TGA AAG CCG ATG TG	103	58
Peroxisome proliferative activated receptor gamma	PPARG	NM_138712	GCC TTG CAG TGG GGA TGT CTG CCT CGC CTT TGC TTT GGT CAG	194	61
Type Iα1 collagen	COL1A1	NM_000088	CGA TGG CTG CAC GAG TCA CAC CAG GTT GGG ATG GAG GGA GTT TAC	180	62
Type IIα1 collagen	COL2A1	NM_001844	CCG GGC AGA GGG CAA TAG CAG GTT CAA TGA TGG GGA GGC GTG AG	128	58
Type IXα3 collagen	COL9A3	NM_001853	AAT CAG GCT CTC GAA GCT CAT AAA A CCT GCC ACA CCC CCG CTC CTT CAT	100	55.1

*bp, base pairs.

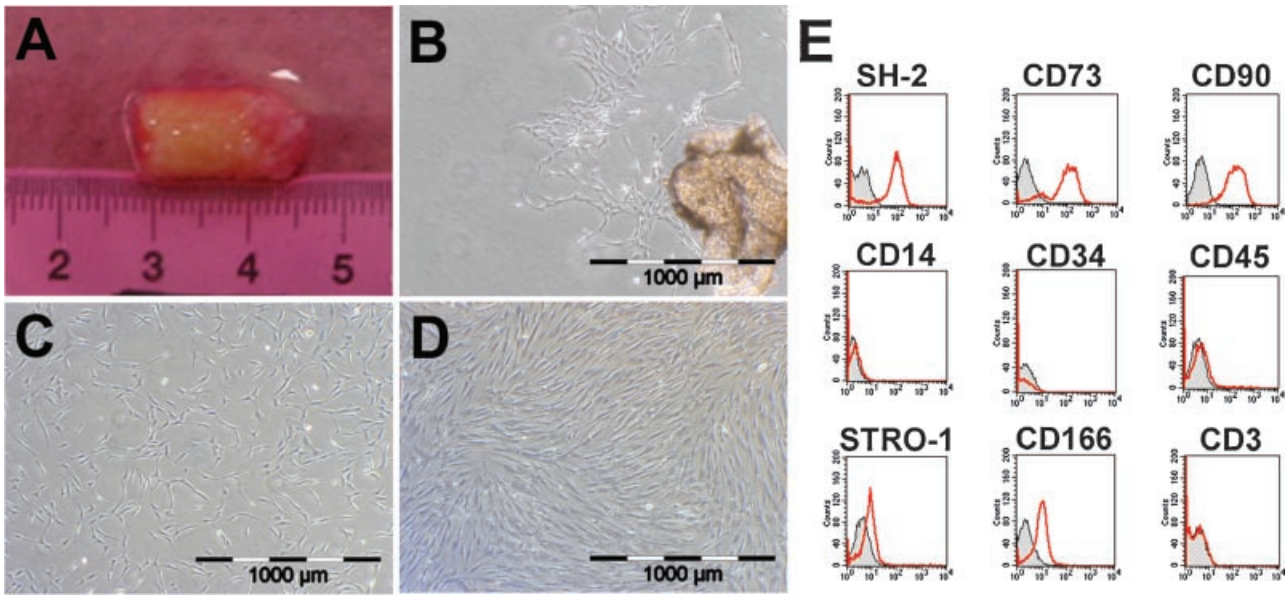

Figure 1. Morphology and cell surface antigen pattern of human cortico-spongious progenitor cells. Macroscopical view of subchondral cortico-spongious bone (A) used for the isolation and outgrowth of progenitor cells (B). CSP cells showed a typical fibroblast-like morphology (C) and a swirl-like pattern when reaching confluency (D). Cells were positive for SH-2, CD73, CD90, CD166, and STRO-1, and negative for CD3, CD14, CD34, and CD45 (E).

cells and proteoglycans (Fig. 2A). Immunohistochemically, cartilage-related type II collagen was detected in the outer layer of the micro-masses derived from four out of five donors (Fig. 2B). The micro-masses derived from one out of five donors showed a dense tissue, rich in proteoglycans, and virtually no type II collagen deposition (data not shown). In contrast, CSP cells not stimulated with TGFβ3 evolved a fibrous tissue, forming neither an extracellular matrix rich in proteoglycan (Fig. 2C) nor type II collagen (Fig. 2D).

Since type II collagen was detected immunohistochemically as early as day 14 (data not shown), semi-quantitative gene expression analysis of genes coding for typical cartilage matrix molecules was performed on that day (Fig. 3). High-density micro-masses of CSP cells stimulated with 10 ng/ml TGFβ3 showed an increased expression of *type IIα1 collagen* (57-fold), *type IXα3 collagen* (370-fold), *cartilage oligomeric matrix protein* (*COMP*, 199-fold), *cartilage link protein* (203-fold), and *aggrecan* (33-fold) in comparison with controls. *Type Iα1 collagen* was not differentially expressed in micro-masses treated with TGFβ3 compared to controls (1.5-fold).

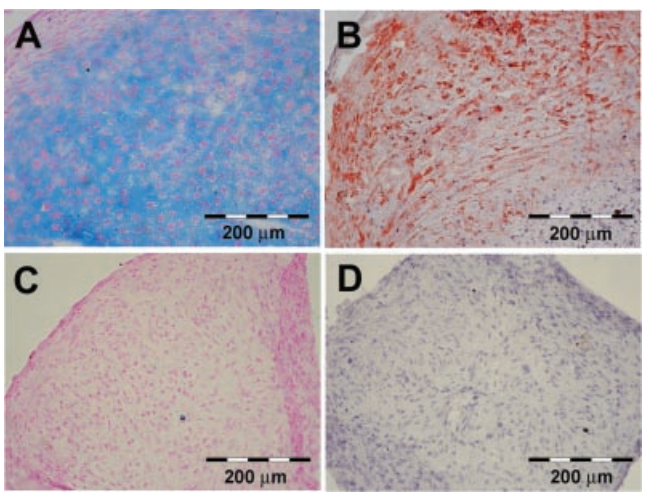

Figure 2. Histological analysis of CSP cells undergoing chondrogenic differentiation. At day 28, CSP cells evolved a compact tissue rich in proteoglycan (A) and type II collagen (B) when cultured in the presence of TGFβ3. Controls not treated with TGFβ3 showed no proteoglycans (C), and type II collagen was not evident (D).

Osteogenic and Adipogenic Differentiation of Human CSP Cells

The potential of CSP cells to undergo osteogenic and adipogenic differentiation was documented by visualization of alkaline phosphatase (AP) activity, staining according to von Kossa and by Oil Red O staining (Fig. 4). At day 28, activity of AP was detected in CSP cells stimulated with osteogenic supplements (Fig. 4A) as well as in nonstimulated controls, to a slightly lower extent (Fig. 4B). Mineralized extracellular matrix components were evident in CSP cells after osteogenic (Fig. 4C) and nonosteogenic stimulation (Fig. 4D), at day 28. Oil Red O staining showed that lipid-filled vesicles were present in both, CSP cells that were cultured under adipogenic (Fig. 4E, arrowhead) or nonadipogenic conditions (Fig. 4F, arrowhead).

Gene expression analysis of typical genes known from the osteogenic (Fig. 5A) and adipogenic (Fig. 5B) developmental sequence showed that the expression of *type Iα1 collagen* was not altered in osteogenic or nonosteogenic cultures compared to confluent CSP cells at day 0. Instead, at days 7 and 28, the expression of *osteocalcin* was induced up to sixfold in osteogenic or nonosteogenic cultures compared to confluent CSP cells. At day 15, the

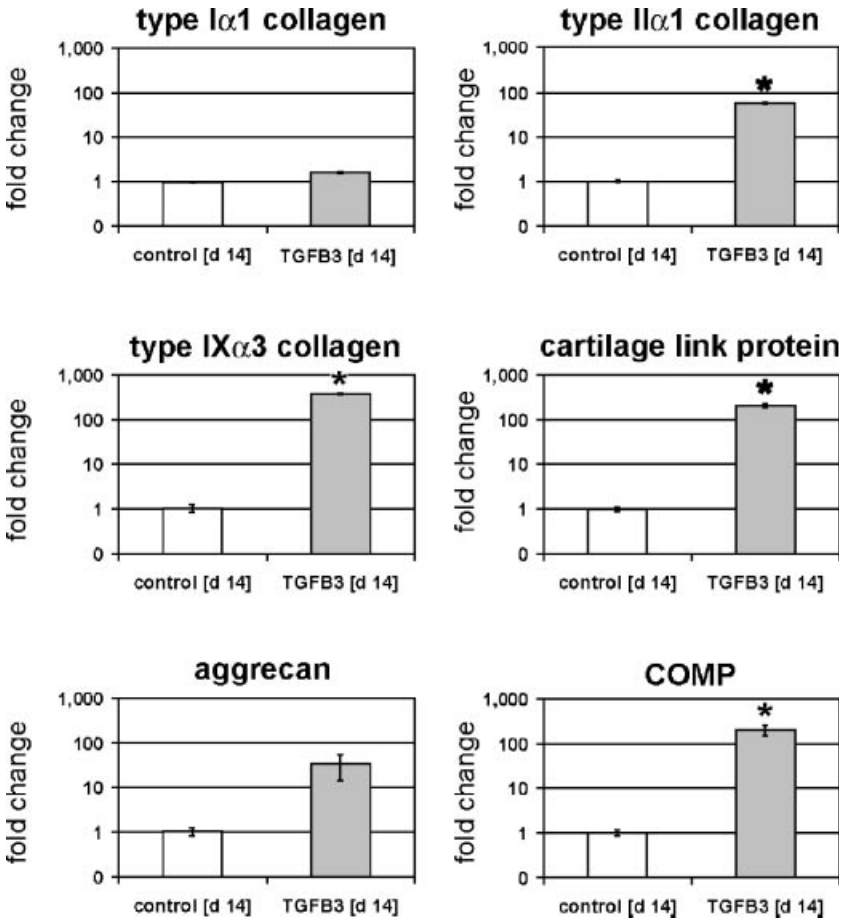

Figure 3. Real-time gene expression analysis of typical chondrogenic marker genes. The gene expression of the chondrocytic marker genes *types Iα1*, *IIα1*, and *IXα3 collagens*, *cartilage link protein*, *aggrecan,* and *cartilage oligomeric matrix protein (COMP)* in chondrogenic CSP cells is given as fold change compared to nonchondrogenic controls. The mean of each triplicate well is plotted and the error bars represent SD. Asterisks indicate significant difference compared to control with $p < 0.05$.

expression of *peroxisome proliferative activated receptor γ (PPARγ)* and *fatty acid binding protein 4 (FABP4)* was induced (15-fold and 4-fold) in CSP cells stimulated with adipogenic medium compared to nonstimulated cells at day 0. Controls, not stimulated with adipogenic medium, showed no differential expression of *PPARγ* or *FABP4*.

DISCUSSION

In the present study, we demonstrated that human mesenchymal cells derived from cortico-spongious subchondral bone marrow and grown in the presence of human serum present the typical cell surface antigen pattern known from human mesenchymal stem cells derived from bone marrow aspirates. The subchondral cortico- spongious bone-derived progenitor (CSP) cells have been shown to have an intrinsic osteogenic differentiation capacity and show low adipocytic development when stimulated with adipogenic medium containing insulin. In contrast, stimulating CSP cells with TGFβ3 strongly induced chondrogenic lineage development with induction of typical chondrogenic marker genes and deposition of cartilage matrix molecules like type II collagen and proteoglycan. This suggests that progenitors from the subchondral bone that are released by microfracture have a prominent chondrogenic differentiation potential. Additionally, chondrogenic CSP cells are promising candidates for cartilage repair approaches.

As shown here, human subchondral CSP cells grown in the presence of human serum present the typical mesenchymal stem cell (MSC) related cell surface antigen pattern[13,22] and are positive for SH-2/CD105, SH-3/CD73, CD90, and CD166. As known from bone marrow aspirate-derived stem cells, CSP cells are negative for the antigens CD14, CD34, and CD45, which distinguishes them from hematopoietic precursors.[23] Interestingly, STRO-1 was only detected in a minority of CSP cells. Although STRO-1 is considered as a marker for MSC,[24] the role of STRO-1 as a stem cell marker is discussed controversially. MSC have been shown to have an expression frequency of STRO-1 between 5% and 13%.[25] In contrast, it has been reported that a subpopulation of adult MSC from bone marrow are negative for STRO-1.[26] However, compared with other cell types, no unique cell surface antigen pattern for MSC exists. MSC show mesenchymal, epithelial, endothelial, and muscle cell surface markers. In addition, it has been reported that single cell-derived colonies of MSC contain at least three different cell types that all show a multipotent developmental potential and differ in their expression of distinct cell surface marker proteins.[26,27] Taking this into account, the cell surface antigen pattern of human subchondral CSP cells as known from

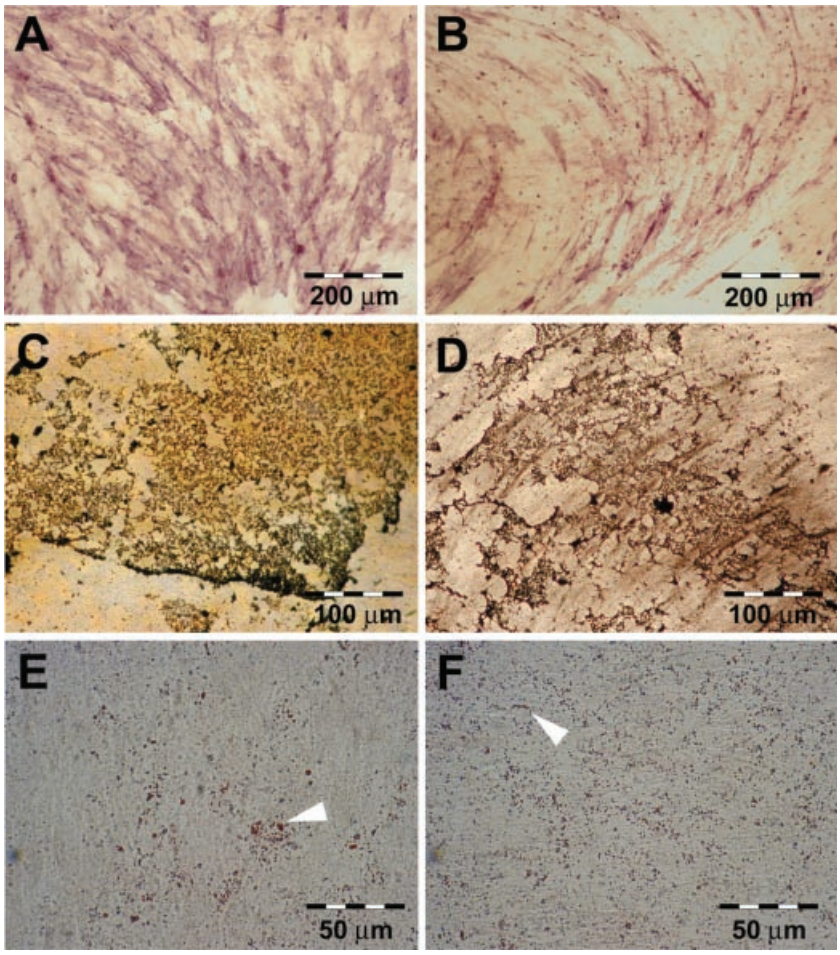

Figure 4. Histological analysis of CSP undergoing osteogenic/adipogenic differentiation. By day 28, osteogenic and nonosteogenic CSP cells showed alkaline phosphatase activity (A,B) and presence of mineralized matrix components according to von Kossa (C,D). By day 15, lipid-filled vesicles (arrowheads) were evident in adipogenic (E) and nonadipogenic (F) CSP cells.

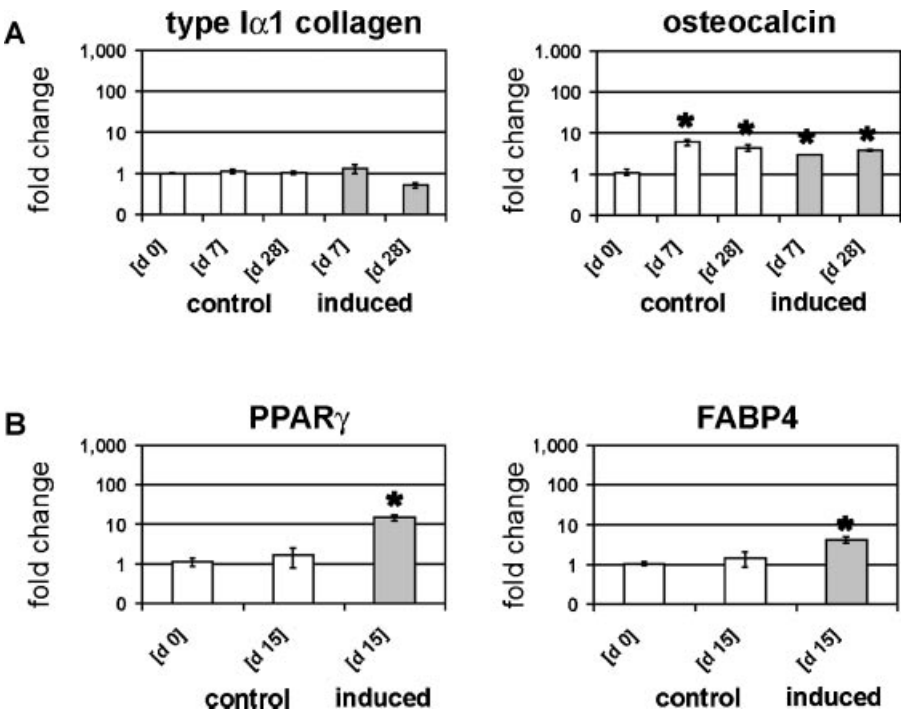

Figure 5. Real-time gene expression analysis of typical osteogenic and adipogenic marker genes. The gene expression of osteogenic (A) marker genes *type I α1 collagen* and *osteocalcin* as well as the analysis of the adipogenic (B) marker genes *peroxisome proliferative activated receptor γ* (*PPARγ*) and *fatty acid binding protein 4* (*FABP4*) is given as fold change compared to untreated CSP cells at day 0. The mean of each triplicate well is plotted and the error bars represent SD. Asterisks indicate significant difference compared to day 0 with $p < 0.05$.

microfracture suggests that these cells are similar to human adult mesenchymal stem cells derived from bone marrow aspirates and represent mesenchymal stem cell-like progenitors.

The stem cell-like character of the subchondral cortico-spongious bone cells is further underlined by the multipotential differentiation capacity of CSP cells. CSP cells showed an inherent osteogenic differentiation capacity with induction of osteocalcin and mineralization of the extracellular matrix, independent of osteogenic stimulation of the cells. Adipogenic induction of CSP cells was accompanied by induction of PPARγ, but not by an increased number of lipid-filled adipocytes. Since PPARγ is known as a key molecule in early adipogenic development[28] and few lipid-filled adipogenic cells were evident, it is most likely that CSP cells underwent adipogenic differentiation when stimulated with adipogenic medium containing insulin. Of special interest for microfracture and, potentially, cell-based cartilage repair approaches, is the chondrogenic differentiation capacity of subchondral CSP cells. Stimulation of CSP cells in high density with TGFβ3 under serum-free conditions induced a variety of typical chondrogenic marker genes including type II collagen. TGFβ3 is a well-known and strong inductor of chondrogenic lineage development, therefore, the main limitation of our study with human subchondral cortico-spongious bone-derived cells is that an appropriate animal study is needed to prove that these multipotent progenitors develop into chondrocytes in vivo and are able to form cartilaginous tissue. However, our in vitro data presented here are consistent with the chondrogenic developmental capacity of human MSC derived from bone marrow aspirates. Meanwhile, chondrogenesis of human MSC was reported after stimulating progenitors with all isoforms of TGF[20,29,30] and selected bone morphogenetic proteins (BMP) including BMP2, BMP6, and BMP9.[31–33] In addition, multilineage developmental potency is not only restricted to MSC derived from bone marrow aspirates. As shown here for subchondral CSP cells, multipotency was also shown for a variety of mesenchymal cells including adipose-derived cells, periosteal cells, synovial stem cells,[25] and osteogenic cells derived from the trabecular bone.[34,35] Especially, those stem cell-like progenitors derived from the trabecular bone have a good osteogenic differentiation capacity. Osteogenic lineage development induced by typical osteogenic supplements, e.g., dexamethasone and β-glycerophosphate, was accompanied by the induction of typical osteogenic marker genes like osteocalcin and bone sialoprotein.[34,35] In contrast, CSP cells mineralized, showed alkaline phosphatase activity, and expressed but did not induce osteogenic markers upon stimulation with typical osteogenic supplements. This may indicate that CSP cells have an inherent osteogenic differentiation capacity. CSP cells may be committed along the osteogenic lineage but may still have the capacity to undergo adipogenic and chondrogenic differentiation.

Recently, it has been shown that MSC can be recovered from a porcine collagen scaffold that was used during the microfracture procedure. After introducing perforations in the subchondral bone, the cartilage defect was covered with the collagen scaffold. The scaffold was allowed to soak with blood for 5 min and a surplus of the collagenous matrix was used for the isolation of multipotent cells presenting typical MSC cell surface antigens.[36]

In microfracture, it is suggested that undifferentiated mesenchymal progenitors enter the defect, proliferate, differentiate to chondrocytes, and synthesize a new matrix containing type II collagen. At 6–8 weeks after injury, the repair tissue within the defect contains chondrocyte-like cells embedded in a matrix consisting of type II collagen, proteoglycans, some type I collagen, and noncollagenous proteins.[37,38] It is most likely that undifferentiated mesenchymal progenitor cells from the blood and/or from the subchondral bone tissue populate the cartilage defect, but factors that initiate the healing sequence and induce progenitors to form cartilaginous repair have still to be elucidated. These repair tissue formation-inducing factors may be released from the bone matrix. Bone is rich in growth factors like TGF and BMPs that may contribute to cell migration and repair tissue formation.[37,38] In addition, synovial fluid with its high content of hyaluronic acid may promote repair tissue formation. Synovial fluid and hyaluronic acid have been shown to initiate chondrogenesis of mesenchymal stem cells derived from bone marrow[39] and to stimulate cartilage healing in the acute period following injury as shown in chick limb bud assays.[40]

In conclusion, multipotent mesenchymal progenitor cells reside in a variety of mesenchymal tissues, including the subchondral cortico-spongious bone that is accessed by microfracture. These cells, as shown here, are stem cell-like mesenchymal progenitors, have the capacity to undergo chondrogenic lineage development, may be guided into the defect area by synovial fluid[19] or human serum,[41] and may contribute to the formation of a cartilaginous repair tissue after microfracture treatment.

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