OsteoArthritis and Cartilage (2006) **14,** 938–943

doi:10.1016/j.joca.2006.02.014

Osteoarthritis and Cartilage

International Cartilage Repair Society

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Brief report
STRO-1$^+$ mesenchymal precursor cells located in synovial surface projections of patients with osteoarthritis

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Summary

Objective: To investigate the presence of mesenchymal precursor cells (MPCs) in synovial surface projections of patients with osteoarthritis (OA), to characterize their phenotype and to show their localization.

Methods: Progenitor cells in synovial surface projections were identified by immunohistochemistry, morphometric analysis and confocal laser scanning microscopy using the following phenotypic markers: STRO-1, CD34, and alpha smooth muscle actin (α-SMA).

Results: In the synovial tissue of all 21 patients with OA MPCs were detected. Immunohistochemistry and subsequent morphometric analysis showed that approximately twice as many STRO-1$^+$ cells/mm^2 were observed in synovial tissue of patients with OA as compared to healthy organ donors and that number of STRO-1$^+$ cells/mm^2 correlated with total cell number/mm^2. Interestingly, in the synovial tissue of patients with OA, twice as many STRO-1$^+$ cells/mm^2 were found in synovial surface projections as compared to the sublining area without villi. Using confocal laser scanning microscopy two populations of STRO-1$^+$ MPCs could be detected in synovial surface projections. Single STRO-1$^+$ cells that co-expressed α-SMA resemble a population of pericyte precursors required to stabilize the immature vasculature. The second STRO-1$^+$ cell population that was found lacked α-SMA but co-expressed CD34 on their surface with low intensity.

Conclusion: Here we can show that in the synovial tissue of patients with OA twice as many STRO-1$^+$ MPCs can be found in synovial surface projections as compared to the sublining area. These cells are preferentially located at the basis and in the protruding end of the synovial surface projection.

Key words: STRO-1$^+$ mesenchymal precursor cells, Villous projections, Synovial membrane, Osteoarthritis.

Introduction

Osteoarthritis (OA) is a degenerative disease which results from aging and abnormal biomechanical stress loading on cartilage and subchondral bone. The synovial membrane can be normal in appearance, showing no intimal hyperplasia or cellular infiltrate, though there can be fibrosis, increased vasculature, and incorporation of fragments of cartilage and bone from the joint surface. A number of cases with OA, however, present features of a noninfectious inflammatory condition with chronic synovitis including intimal cell hyperplasia, infiltration of lymphocytes, plasma cells and cells from the monocyte/macrophage lineage and synovial surface projections. During degenerative and/or noninfectious inflammatory processes in OA, the damaged articular cartilage and bone can be replaced by newly differentiated cells derived from progenitor cells or by self-repair induced by fully differentiated resident cells of the joint[1,2]. Mesenchymal precursor cells (MPCs) are under intense investigation for their high regenerative potential and their ability of self-renewal and differentiation into various connective tissue lineages, i.e. chondroblasts, osteoblasts, adipocytes and smooth muscle cells[2–5]. Mesenchymal cells co-expressing bone morphogenetic protein receptor (BMPR) IA and BMPR II but not CD34 could be successfully generated in primary cultures from cells isolated from joint effusions of patients with rheumatoid arthritis (RA) and other forms of inflammatory arthritis[6]. Mesenchymal cells were also found in the intimal lining layer and in the subintima of the synovial tissue, scattered in areas with small blood vessels and lymphoid aggregates[6–8]. Recently we have shown that MPCs within the synovial sublining area of patients with RA and OA express STRO-1 on their cell surface, and that they reside in close proximity to CD133$^+$ and CD34$^+$ progenitor cells[9]. These cells form cell clusters, where STRO-1$^+$ MPCs were found on the outside of the cluster together with CD133$^+$ precursor cells surrounding CD34$^+$ progenitor cells in the center. MPCs isolated from synovial membranes, obtained from adult human joints, could be expanded *in vitro* and maintained their multilineage differentiation potential[8,10]. MPCs from joint effusions and from bone marrow aspirates showed a similar phenotype and comparable capacity to form colony forming units-fibroblasts, and MPCs in the synovial fluid were more numerous in patients with OA than in healthy individuals[11].

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Received 7 December 2004; revision accepted 28 February 2006.

It seems, that in the joint, resident MPCs located in particular microenvironments known as niches, have a lifelong capability to replace impaired or lost cell populations and counteract degeneration[2]. On the other hand, MPCs might also be responsible for extensive synovial connective tissue proliferation and formation of villous projections as seen in OA. Here we investigate whether MPCs can be found in synovial surface projections of patients with OA and try to characterize their phenotype. It was speculated that synovial villi do not form simply by outgrowth, but rather by tissue splitting, surface bridging and subintimal cavitation[12].

Material and methods

PATIENTS

Synovial tissue of 21 OA patients was obtained at the time of surgery for knee and hip arthroplasty after informed consent was obtained. Patients with OA fulfilled the respective classification criteria[13]. Of the 21 OA patients, 14 had knee surgery with a mean Knee Society Score of 42 points (SD 18, range 9–64)/46 points (SD 19, range 10–80), and seven had hip surgery with a mean Harris Hip Score of 47 points (SD 4, range 42–55). Synovial tissue of 11 joints from three organ donors was obtained in accordance with the European Society for Organ Transplantation and with the Austrian Hospital Act (KAG 1982, §62 a, b, c).

IMMUNOHISTOCHEMISTRY AND MORPHOMETRIC ANALYSIS

Sections (5 μm) of snap-frozen synovial membrane samples from patients with OA and organ donors were prepared and immunohistochemistry performed as previously reported[9]. Acetone-fixed sections were incubated overnight at 4°C with the following mouse monoclonal antibodies diluted in Tris buffered saline (TBS)/1% bovine serum albumin (BSA): STRO-1 (1.8 μg/ml; Developmental Studies Hybridoma Bank, IA), CD34 (0.5 μg/ml; Immunotech, Marseille, France), VEGFR-2 (2.5 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA), CXCR4/Fusin (2.5 μg/ml; PharMingen, San Diego, CA) and anti-muscle specific actin α and γ [alpha smooth muscle actin (α-SMA)] (0.5 μg/ml; Sigma, St. Louis, MO). The reactivity of primary antibodies was revealed using biotinylated sheep $F(ab')_2$ anti-mouse immunoglobulin (Ig) (1:100; An der Grub, Kaumberg, Austria) diluted in 500 μg/ml normal human Ig (Intraglobin F, Biotest, Dreieich, Germany) followed by streptavidin–horseradish peroxidase (HRP) complex (1:100; Extravidin–HRP, Sigma). Endogenous peroxidase was blocked during the incubation with secondary antibody by adding D-glucose and glucose-oxidase type VII. For the detection of STRO-1 and CXCR4 mAb binding, a super-sensitive detection system consisting of biotinylated goat anti-mouse Ig and streptavidin–HRP (Biocare Medical, Carlsbad, CA) was used. Omission of primary antibody and isotype controls was included in the protocol. The sections were exposed to 3-amino-9-ethylcarbazole (AEC; Sigma) and counterstained with Mayer's Hemalum. Morphometric analysis was performed by counting the number of STRO-1^+ cells as well as the total number of cells in 10 fields of 1 mm^2 selected at random as described earlier[9]. Student's *t* test was used to evaluate differences in the number of STRO-1^+ cells/mm^2 in the synovial surface projection compared to the number of STRO-1^+ cells/mm^2 in the sublining area without villi. Differences were considered statistically significant when $P < 0.05$.

THREE-COLOR LASER SCANNING CONFOCAL MICROSCOPY

For immunofluorescence studies, acetone-fixed synovial tissue sections (5 μm) were prepared, incubated overnight at 4°C with STRO-1 (1.8 μg/ml) diluted in TBS/1%BSA. Staining of the mouse IgM mAb STRO-1 was revealed using a biotin-conjugated goat anti-mouse IgM (2.5 μg/ml; Sigma) followed by a streptavidin–Cy5 complex (2 μg/ml; Jackson ImmunoResearch, West Grove, PA). After blocking tissue sections with normal mouse serum (1:5), they were incubated with anti-α-SMA (1 μg/ml; Neo-Markers Inc., Fremont, CA) labeled with Alexa-Fluor 594 (Molecular Probes Inc., Eugene, OR) and with CD34 FITC (5 μg/ml; Becton Dickinson, San Jose, CA) overnight at 4°C. Serial dilutions of each primary and secondary antibody were tested to minimize nonspecific binding, assure separation of the fluorescent signals, and optimize fluorophore concentration to preclude self-quenching. Sections were analyzed with a laser scanning confocal microscopy (LSM 510; Zeiss, Oberkochen, Germany) with multiphoton laser (argon-laser: 488 nm for FITC, HeNe 1: 585 nm for AF594, and HeNe 2: 633 nm for Cy5) and a 63× Zeiss Plan-Apochromat DIC oil immersion objective with numerical aperture 1.40.

Results

Of the 21 patients with OA investigated, 13 patients showed synovial surface projections. Eight of them had massive lymphocyte infiltration, while in five modest or no signs of lymphocyte ingress were found. Two patients showed lymphocyte infiltration, without synovial surface projections, and in the synovial tissue of the remaining six patients no synovial villi and hardly any leukocyte infiltration were found. Morphometric analysis showed that, although not statistically significant, approximately two times more STRO-1^+ cells/mm^2 were observed within the synovial sublining area of patients with OA as compared to healthy organ donors (Table I). In both groups the number of STRO-1^+ cells/mm^2 correlated positively with total cell number/mm^2. Interestingly, twice as many STRO-1^+ cells/mm^2 were found in synovial surface projections as compared to the sublining area without villi (Table I, the

Table I
Morphometric analysis of total cells and STRO-1^+ cells/mm^2 in the sublining area of the synovial tissue and in synovial surface projections of patients with OA

	Total cells/mm^2	STRO-1/mm^2 in the sublining area	STRO-1/mm^2 in synovial surface projection
Patients with synovial surface projections ($n = 13$)	304 ± 120	5.4 ± 3.3*	12.1 ± 4.7*
Patients without synovial surface projections ($n = 8$)	208 ± 81	4.8 ± 4	–
Healthy controls ($n = 11$)	128 ± 13	2.4 ± 1.3	–

Results are given as mean ± SD, and the Student's *t* test was used to evaluate differences in the number of STRO-1^+ cells/mm^2 in the sublining area compared to the number of STRO-1^+ cells/mm^2 in synovial surface projections, obtained by morphometric analysis. Differences were considered statistically significant when $P < 0.05$, indicated by an asterisk.

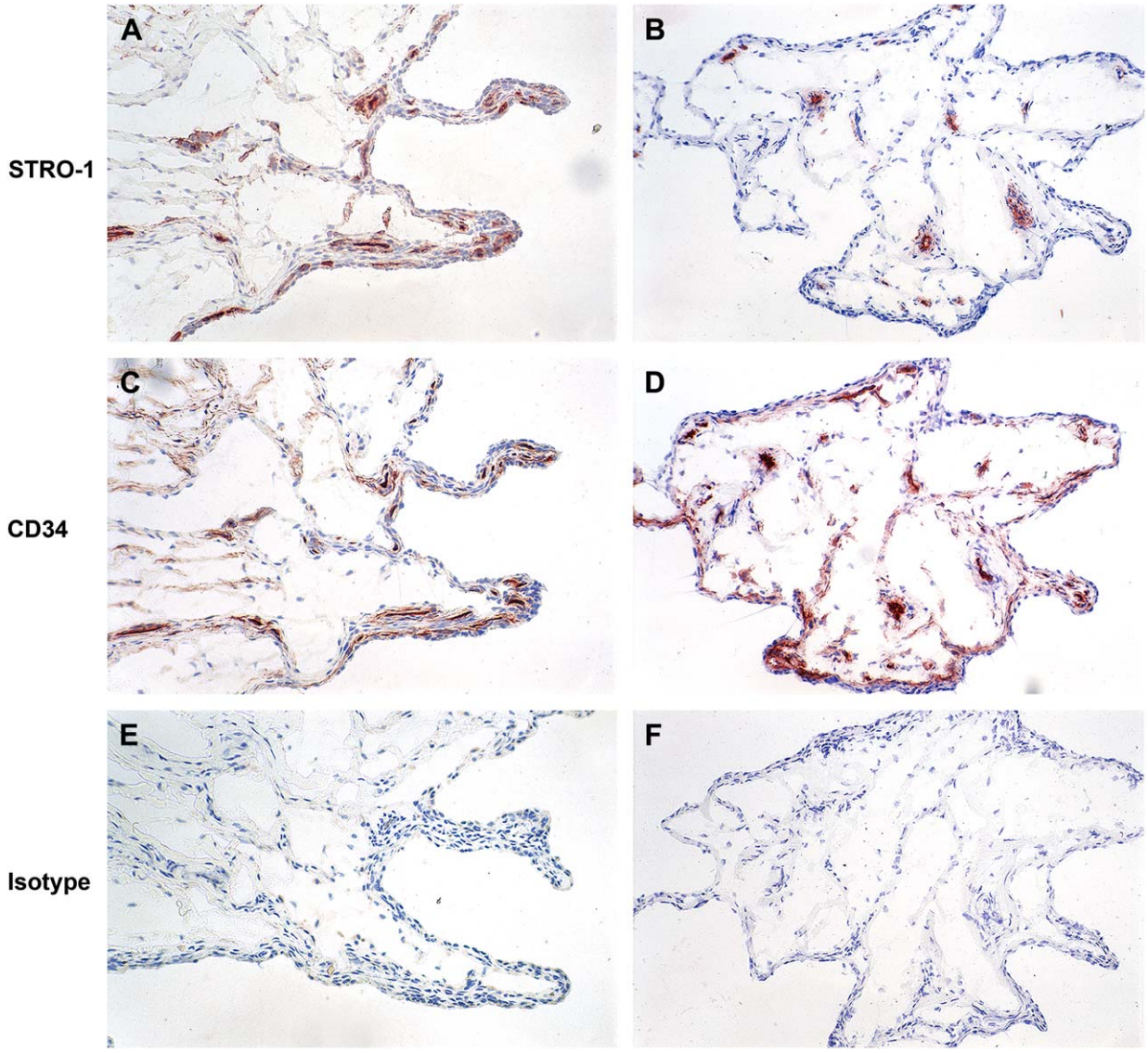
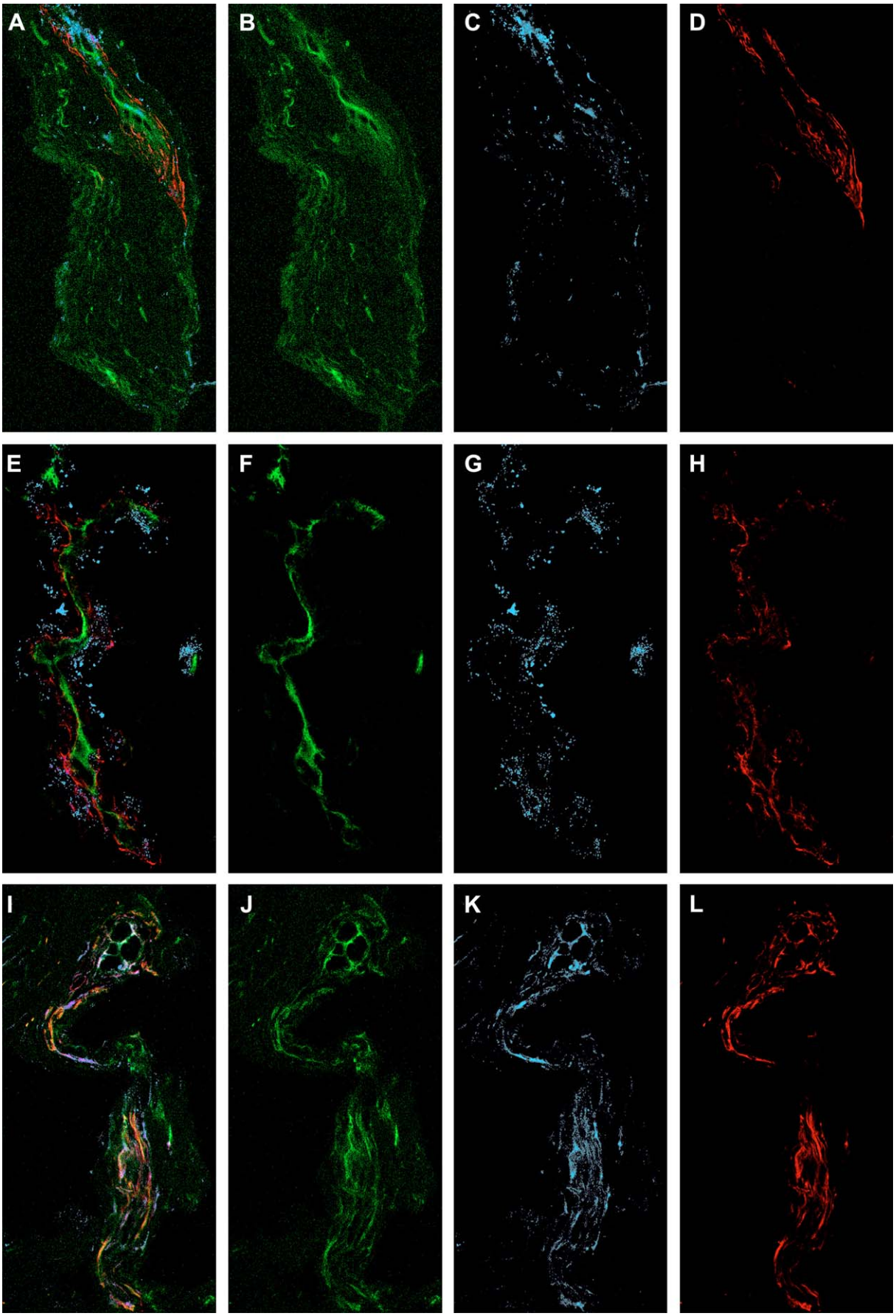


Fig. 1. Expression of STRO-1 and CD34 on precursor cells in serial sections of synovial tissue obtained from two representative patients with OA. Serial sections of synovial membranes from patient 1 (A,C,E) and patient 2 (B,D,F) were stained with the mesenchymal precursor cell marker STRO-1 (A,B), with the endothelial and stem cell marker CD34 (C,D) and with an isotype control (E,F), and were counterstained with Mayer's Hemalum. The two images were selected to show a synovial surface projection longitudinal (A,C,E) and transversal (B,D,F) (original magnification $\times$150).

statistical difference is indicated with an asterisk). Serial longitudinal cross sections of a synovial surface projection of one representative patient with OA are shown in Fig. 1(A,C,E), where STRO-1$^+$ cells are found in small cell clusters [Fig. 1(A)] surrounding CD34$^+$ precursor cells [Fig. 1(C)]. Figure 1(B,D,F) gives the transversal image of the basis of a synovial surface projection of another representative patient with OA. Here, STRO-1$^+$ cells are scattered within the interior in groups of 3–10 cells surrounding CD34$^+$ precursors [Fig. 1(B,D)]. Immunohistochemistry gives perfect morphologic images, but this technique is not precise enough to answer the question of

Fig. 2. Three-color laser scans showing the expression of STRO-1, CD34 and α-SMA on precursor cells located within a synovial surface projection of a patient with OA. Acetone-fixed synovial tissue sections (5 μm) were incubated with mouse IgM mAb STRO-1 and staining was revealed using a biotin-conjugated goat anti-mouse IgM followed by a streptavidin–Cy5 complex. After blocking tissue sections were incubated with anti-α-SMA labeled with Alexa-Fluor 594 and with CD34 FITC overnight at 4°C. Sections were analyzed with a confocal laser scanning microscope (LSM 510, Zeiss, Oberkochen, Germany) and a differential interference contrast oil immersion objective. Single scans showing precursor cells staining positive for CD34 green (B,F,J), STRO-1 blue (C,G,K) and α-SMA red (D,H,L), and overlays of all three scans are shown in A, E, and I (the magnification of A–D is $\times$630 and of E–L $\times$850).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whether these STRO-1$^+$ cells also co-express the pericyte marker α-SMA, and the endothelial and stem cell marker CD34. Confocal laser scanning microscopy is the perfect tool to demonstrate co-localization of STRO-1, CD34 and α-SMA on the cellular level. Three-color images with CD34$^+$ cells in green, α-SMA$^+$ cells in red and STRO-1$^+$ cells in blue show a synovial surface projection of a patient with OA in longitudinal orientation [Fig. 2(A,B,C,D)], a sublining area of synovial membrane obtained from a patient with OA [Fig. 2(E,F,G,H)], and a sublining area of a healthy individual [Fig. 2(I,J,K,L)]. Interestingly, the majority of STRO-1$^+$ cells [Fig. 2(A,D)] within the synovial surface projections did not express α-SMA [Fig. 2(A,C)], and only a minor STRO-1$^+$ cell population existed that co-expressed α-SMA on the surface, representing the population of pericyte precursors required to stabilize the immature vasculature. Single STRO-1$^+$ precursors co-expressed CD34 on their surface, but with low intensity [Fig. 2(A)]. Certain CD34$^+$ precursors that co-expressed STRO-1 very dimly were also detected, but they represent a rare population [Fig. 2(A) and data not shown]. In the sublining area of the synovial membrane of both patients with OA and healthy individuals, the majority of STRO-1$^+$ cells co-expressed α-SMA and were located in areas of new vessel formation [Fig. 2(E,G,I,K)]. Here single STRO-1$^+$ precursors also co-expressed CD34 on their surface with low intensity.

Discussion

The damaged articular cartilage and subchondral bone can be replaced by newly differentiated cells derived from a limited number of readily available resident multipotent MPCs or by fully differentiated cells of the joint[1–4,14,15]. MPCs resident in small niches in the peripheral tissue, are recruited to differentiate into locally required mature cell types, and are replaced by circulating MPCs which are in a 'steady state' of equilibrium with the resident MPCs in the niches[16]. In the joint of patients with OA MPCs could be found in the synovial tissue, in cartilage and bone and in joint effusions[6–8,11,15]. Within the synovial membrane STRO-1$^+$ MPCs were found in the sublining area in close proximity to CD34$^+$ and CD133$^+$ progenitor cells, that are involved in the *de novo* form of a primitive vascular network[9]. These cells form cell clusters with CD34$^+$ progenitor cells located at the inside surrounded by CD133$^+$ progenitor cells and STRO-1$^+$ MPCs on the outside. STRO-1$^+$ α-SMA$^+$ cells in these clusters show phenotypic features of immature pericytes and/or pericyte precursors and can be recruited from neighboring resident MPCs or migrate to the joint via circulation. Pericytes are needed to coat the newly formed micro-vasculature and stabilize by preventing vessel pruning[17]. In addition to the sublining area of the synovial tissue, STRO-1$^+$ MPCs can also be found in synovial surface projections. Synovial villi were found in 13 of the 21 patients with OA at the time of surgery for arthroplasty. Morphometric analysis revealed that the number of STRO-1$^+$ MPCs/mm^2 were two times higher in synovial surface projections than in the sublining area of synovial membranes without villi. Within these synovial surface projections MPCs are preferentially located at the basis and at the protruding end. Interestingly, the majority of STRO-1$^+$ MPCs did not express α-SMA on their surface and it seems likely that they represent a different population of MPCs as compared to MPCs in the sublining area. Confocal microscopy showed a minor STRO-1$^+$/α-SMA$^-$ MPC population expressing CD34 on their surface, but with low intensity.

The presence of MPCs in the synovial tissue of patients with OA raises the question of whether these cells appear in the joint as a result of inflammatory processes or whether they are already present before the onset of the disease. A murine collagen-induced arthritis model showed that BMPR$^+$ mesenchymal cells are among the earliest cells to arrive at the pre-arthritic joint and their presence in the chronic phase of arthritis is regulated by Tumor Necrosis Factor (TNF)[14]. Further, synovial tissue of healthy organ donors contains MPCs (Table I) and in every peripheral tissue MPCs are found at various quantities with the bone marrow serving as a reservoir[1,4,16]. Both homeostasis and canals between bone marrow and synovium allow migration of bone marrow stem cells into the synovium of inflamed joints[18]. The mechanism of opening up bone canals is at least partly osteoclast-mediated, as activated osteoclasts were found in close proximity to the enlarged canals[18].

In conclusion, we can say that in patients with OA twice as many STRO-1$^+$ MPCs were found in synovial surface projections preferentially at the basis and at the protruding end, as compared to the synovial sublining area without villi. In addition, the number of STRO-1$^+$ MPCs correlated with lymphocyte infiltration. This is an interesting finding since tissue repair activity is assumed to be intense in the synovial tissue of patients with inflamed joints. Differentiated cells of the mesenchymal lineage have a particular capability, they can de-differentiate and then shift to another mesenchymal phenotype, which highlights the reversible nature of differentiation of mesenchymal cells[3,19]. It seems likely that, in OA, MPCs are, at least in part, responsible for extensive synovial connective tissue proliferation and formation of villous projections.

Acknowledgment

We thank Prof DDr R. Mayr for helpful discussion, and Dr M. Groger and Prof Dr P. Petzelbauer for kindly providing the confocal microscope. This work was supported by the project grant P13481 from the Austrian Science Fund (FWF).

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