

The feasibility of the CD271⁺ and CD271⁻ mesenchymal stromal cell enrichment toward nucleus pulposus-like cells

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Abstract

Introduction. Factors promoting nerve cell ingrowth are considered responsible for chronic back pain resulting from the intervertebral disc degeneration (IDD). One of the recent exploratory IDD treatments is stem cell transplantation therapy. The CD271 (low-affinity nerve growth factor receptor) has been identified as a marker of the most homogeneous mesenchymal stem cell (MSC) subset. It is capable of promoting differentiation along adipogenic, osteogenic and chondrogenic lineages and producing significantly higher levels of cytokines as compared to the total population of plastic adherence-mesenchymal stem cells (PA-MSCs).

We investigated the ability of CD271⁺ MSCs to differentiate into chondrocyte-like cells of the nucleus pulposus (NP) of intervertebral disc. We also examined CD271⁻ MSCs, using PA-MSCs as a control cell population.

Material and methods. Bone marrow derived PA-MSCs and its two subsets, CD271⁻ MSCs and CD271⁺ MSCs, were seeded in collagen scaffolds. After two weeks of growth in NP-differentiation medium, RNA was isolated from cells-scaffold constructs and was analyzed by q-PCR for expression of NP markers. Glycosaminoglycans were analyzed biochemically directly in cells-scaffold constructs.

Results. Expression of NP markers — extracellular matrix components such as aggrecan, collagen type II and glycosaminoglycans on both RNA and the protein levels — was significantly higher in CD271⁻ MSCs compared to the CD271⁺ MSCs and PA-MSCs cell populations.

Conclusions. CD271⁻ MSCs may be superior candidates for NP restorative treatment compared to CD271⁺ MSCs and PA-MSCs due to their ability of expressing NP-supporting extracellular matrix components at levels higher than the other two studied MSC subsets. (*Folia Histochemica et Cytobiologica* 2017, Vol. 55, No. 3, 114–123)

Key words: mesenchymal stem cells; CD271; intervertebral disc regeneration; chondrocyte-like cells; differentiation

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Introduction

The exact mechanisms of the intervertebral disc degeneration remain unclear. Pathological changes observed during the disc degeneration process have both genetic and environmental background and are often related to the spine mechanics combined with traumatic injury. Following intervertebral disc (IVD) degeneration, the biomechanical status of the vertebral column is altered, thereby increasing the probability of the facet joint degeneration, spondylosis, spondylolisthesis, and spinal stenosis. These pathologic processes are the main reason for an acute or chronic low back or neck pain. Discopathy is also responsible for the substantial biochemical changes in the disc structure. The necessity to better understand the pathological phenomena behind biochemistry and cell biology of intervertebral discs spurred the research on stem cells and the regenerative stem cell transplantation as a new potentially curative treatment for IVD [1–3].

To date, mesenchymal stem cells (MSCs) are most frequently used in cell-based therapies due to the ease of their availability and suitability in autologous transplantations. The best-fit definition of mesenchymal stem cells points to their multipotent, stromal phenotype with the ability to differentiate into cells of mesenchymal origin such as bone, cartilage and adipocytes. MSCs show a high capacity to adhere to plastic surfaces [4] and are typically defined by the expression of CD73, CD90, CD105, and lack of expression of hematopoietic markers and MHC class II antigens [5]. However, MSCs constitute a very heterogeneous population of multipotent progenitor cells with a varying proliferative, differentiation and immunosuppressive potential that differ in the sets of surface markers. These variations relate both to the different MSC sources such as bone marrow, adipose tissue and Wharton jelly, and to the potential for differentiation. For instance, in some studies the differentiation along adipogenic, osteogenic and chondrogenic lineages was observed in only about one-third of bone marrow-derived MSC (BM-MSC) clones, while most clones (60–80%) displayed only osteo- or chondrogenic potential [6]. Other studies have demonstrated this trilineage differentiation ability for only about 50% of colony-forming cells [7]. On the other hand, only about 7% of BM-MSCs were able to differentiate toward multiple lineages, when their phenotype was tested at a single-cell level [8]. Interestingly, tri-, bi-, and uni-directional clones were obtained from 100 single-cell derived clones, confirming that MSCs are composed of cells with different

differentiation potential [9]. Mareddy and coworkers demonstrated the presence of fast- and slow-growing MSC clones, where the fast-growing clones were tripotent, and slow-growing clones displayed limited differentiation ability and often showed morphological features associated with cellular senescence [10].

Efforts aimed at generation of bio-materials for the regenerative stem cell-based therapies focus on the search for markers or combination of markers allowing isolation of cell populations optimized for their subsequent use in transplantation.

According to several recent reports [11–13], expression of CD271 is associated with the most homogeneous MSC subset capable of differentiating along adipogenic, osteogenic and chondrogenic lineages, and of producing significantly higher levels of cytokines than plastic adherence-mesenchymal stromal cells (PA-MSCs) [14–17]. CD271 was initially described as a nerve growth factor receptor (NGFR); it is also known as LNGFR (low-affinity nerve growth factor receptor), and belongs to the low-affinity neurotrophin receptor family and to the tumor necrosis factor receptor superfamily [18, 19]. CD271 interacts with neurotrophins such as brain-derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4, and with pro-neurotrophins (proNTs), including proNGF and proBDNF [18–20]. This antigen is expressed by several cell types, including neurons, Schwann cells, mesenchymal stem cells, follicular dendritic cells, and melanocytes among other cell types [19, 22, 23]. While present on cells of the nervous system, CD271 is thought to be involved in the development, survival, and differentiation of neural cells [19–22]. CD271⁺ MSCs demonstrated also higher cell proliferation capacity compared to the entire PA-MSC population [23]. However, CD271 has been also identified as a marker of tumor initiating cells that may affect cell survival and proliferation [24–26].

The finding that the CD271⁺ MSCs can effectively differentiate towards cartilage tissue [15, 27, 28] has inspired us to investigate the ability of these cells to differentiate into chondrocyte-like cells of the nucleus pulposus (NP) of IVD. We have also investigated CD271⁻ MSCs and compared these cells to the entire population of MSCs generated by plastic adherence [29–31]. Based on the gene and the glycosaminoglycans (GAG) expression profiles characteristic for healthy NP, we hypothesized that the CD271⁻ MSCs may be valuable candidates for NP restorative treatment. To the best of our knowledge, this is the first study in which chondrocyte-like phenotypes of both CD271⁺ MSCs and CD271⁻ MSCs have been compared.

Material and methods

Processing of bone marrow and cultures of MSCs. All patients gave an informed written consent and research was carried out in compliance with the Helsinki Declaration. The experimental design was approved by the Ethic Committee of the University of Warmia and Mazury, Olsztyn, Poland. Bone marrow aspirates were obtained from patients undergoing iliac bone graft or total hip replacement ($n = 5$, 2 females, 3 males; median age 53 years, range: 40–70 years) using heparin-rinsed trocar and syringe; none of the subjects had underlying diseases.

In addition to the population of plastic adherence-mesenchymal stem cells (PA-MSCs) [4], CD271-positive and CD271-negative cell subsets were selected. The CD271⁺ subset of MSCs were isolated using immunomagnetic method based on MACSelect CD271 (LNGFR) Microbeads kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturer's recommendations and described as CD271⁺ MSCs. Cells remaining after this isolation step were considered as CD271 negative and referred to as CD271⁻ MSCs. Briefly, a phosphate-buffered saline (PBS)-diluted cell fraction of bone marrow was layered over a Ficoll density gradient (1.077 g/mL, GE Healthcare, Little Chalfont, UK), followed by centrifugation at 400 g at room temperature for 40 min. Nucleated cells were collected, diluted with two volumes of PBS, centrifuged twice at 100 g for 10 min, and finally resuspended in DMEM/F-12, GlutaMAX(TM) with glucose (3151 mg/L) (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA) and 1% 10,000-U penicillin/streptomycin (P/S) (Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37°C in a 5% CO₂ atmosphere in a T-75 flask (Becton Dickinson, New Jersey, NJ, USA). After 5 days, the medium was replaced and unattached cells were removed. Thereafter, the medium was replenished every 2–3 days, and, after reaching confluency at about 14 days in culture, the cells were digested with 0.05% trypsin/ethylenediaminetetra acetic acid (EDTA) (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) for microencapsulation. In the first step of cell isolation using a Ficoll density gradient, usually about 1×10^8 of mononuclear cells were obtained, and in the next step in which immunomagnetic method was applied, about 2% cells positive for CD271 marker were obtained and the remaining 98% were CD271⁻ cells. This yield of CD271⁺ cells was consistent throughout all cell purification experiments ($n = 5$).

Immunocytochemical characterization of the analyzed cell populations. The phenotype of MSCs was confirmed immunocytochemically [32] using commonly recognized positive (CD90, CD73) as well as negative (CD45, CD34) surface markers of stemness.

Samples in 24-well culture plate were rinsed briefly in 0.1 M PBS and fixed with 4% buffered paraformaldehyde (pH 7.4) for 10 min. Next, MSCs were permeabilized with 0.025% Triton X-100 (Sigma-Aldrich) for 5 min. After this step, those cells were incubated with a blocking mixture containing 10% normal goat serum (Invitrogen) in PBS for 30 min at 37°C to reduce non-specific background staining. In the next step, the MSCs were washed with PBS (3×5 min). After a wash, those cells were incubated with a mixture of primary antibodies for CD90 (mouse anti-human, Becton Dickinson), CD73 (mouse anti-human, Invitrogen), CD45 (mouse anti-human, Becton Dickinson) and CD34 (mouse anti-human, Invitrogen) overnight in the humid chamber at 4°C. Next, MSCs were washed again with PBS (3×5 min) and primary antisera were then visualized by a mixture of secondary antibodies (Alexa Fluor 488 and Alexa Fluor 555; Jackson Immuno Research Labs, Baltimore Pike, PA, USA) for 1 h at room temperature and in the darkness. The working concentration of primary and secondary antibodies was 1:500. After the incubation, those cells were washed again with PBS (3×5 min) and then were mounted with mounting medium with DAPI (Santa Cruz Biotechnology, Dallas, Texas, USA).

Microencapsulation and 3D cultures of mesenchymal stem cell subsets. Rat-tail collagen type I (Becton Dickinson, New Jersey, NJ, USA) was first neutralized by 1 N sodium hydroxide and diluted into a final concentration of 2 mg/mL. CD271⁺ MSCs, CD271⁻ MSCs, and PA-MSCs were suspended in the neutralized collagen solution to make up cell matrix mixtures with initial cell density of 1,000 cells in 5 μ L droplets. Liquid droplets were then dispensed onto a non-adhesive surface of sterile parafilm sheets placed in Petri dishes and incubated at 37°C in a 5% CO₂ atmosphere for 45 min to induce gelation of collagen [33]. The gelated microspheres were then suspended in a chemically defined basal medium containing DMEM supplemented with 10 ng/mL transforming growth factor 1 (TGF-1; R & D Systems, Minneapolis, MN, USA), 100 nmol/L dexamethasone, 50 g/mL ascorbate 2-phosphate, 100 g/mL sodium pyruvate, 40 g/mL proline and ITS-plus (Sigma-Aldrich), and cultured at 2% oxygen using O₂/CO₂ incubator (Sanyo Moriguchi, Osaka, Japan) for 10 days [34].

Measurement of glycosaminoglycans content. To determine the GAG content in the microspheres, a modified 1,9-dimethylmethylene blue (DMMB) dye-binding assay was used [35]. For each population, 30 microspheres were placed in a phosphate buffer at pH 6.5 (50 mM phosphate buffer, 5 mM EDTA, and 5 mM L-cysteine) containing 5 μ L of 20 mg/mL proteinase K (Sigma-Aldrich) at 60°C overnight to solubilize the proteoglycans. Unlike the traditional DMMB assay, 10% propan-1-ol was included in the decomplexation

Table 1. Primers used for RT-PCR analysis

Gene (abbr.)	Forward primer (5'-3')	Reverse primer (5'-3')
Collagen type II (COL2A1)	gaatccagggtcctcaag	accagtgaagccacg
Collagen type I (COL1A1)	ccagcaaatgttccttttg	aaaattcacaagtccccatc
Aggrecan (ACAN)	cagaccgtcagatacc	ataggtcctgaccctg
Keratin 19 (KRT19)	acaacaattgtctgcctc	atthttattggcaggtcagg
Transcription factor (SOX9)	aaatcaacgaaaactggac	atttagcacactgatcacac
Paired box 1 (PAX1)	attaaatacactcagtcggc	atgcacagctacgcc
GAPDH	ctctgatttgctgtattgg	gtaaacatgtagttaggctc
β -actin (ACTB)	gacgacatgagaaaatctg	atgatctgggtcatctctc
RN18S1	atcggggattgcaattatc	ctcactaaaccatccaatcg

solution to enhance DMMB signal at 656 nm. Absorbance was measured using a microplate reader (Infinite 200 PRO, TECAN, Männedorf, Switzerland). To evaluate the amount of GAGs in the samples, calibration curve was constructed using chondroitin 6-sulfate (Sigma-Aldrich) as a standard, and the GAG concentration was determined using its linear region between 0.0625 and 2 mg/100 mL. Filtration of the pre-heated, proteinase K-treated samples eliminated most of the interfering DNA, resulting in the total loss of DNA-DMMB complexes. To avoid possible overestimate of the GAG concentration due to the traces of the DNA-DMMB complexes, all samples of microspheres were filtered following proteinase K treatment.

Measurement of the expression of NP cellular markers by qPCR. Microspheres were homogenized with 1 mL of TRI reagent (Sigma-Aldrich), 100 mL of 1-bromo-3-chloropropane (Sigma-Aldrich) were added to the homogenates and the mixture was shaken vigorously for 15 min. The mixture was phase-separated following centrifugation at 12,000 g for 15 min at 4°C, where RNA remained in the upper aqueous phase while DNA and proteins separated into the interphase and organic phase. RNA was precipitated by adding 500 mL of isopropanol and pelleted by centrifugation at 12,000 g for 10 min at 4°C. The supernatant was removed and RNA pellet was centrifuged at 8000 g for 5 min at 4°C after washing with 1 mL of 75% diethylpyrocarbonate (DEPC)-(Sigma-Aldrich)-treated ethanol. Ethanol was removed and RNA pellets were air-dried for 10–15 min, dissolved in DEPC-treated water and incubated for 15 min at 60°C. Reverse transcription of total RNA to cDNA was performed using the Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Fisher Scientific), containing oligo(dT)₁₈ and random hexamer primers to prime synthesis of first strand of cDNA. The concentration of RNA was 100 ng/ μ L. The RT-PCR conditions were: incubation for 10 min at 25°C followed by 15 min at 50°C, termination the reaction by heating at 85°C for 5 min. To determine the direction of PA-MSCs and their

subsets differentiation the expression levels of genes characteristic for healthy intervertebral disc, including paired box 1 (PAX1), SOX-9, and intercellular matrix proteins: aggrecan (ACAN), collagen type I (COL1), collagen type II (COL2), and keratin 19 (KRT19) were evaluated (Table 1). Real Time PCR was performed using Power SYBR Green PCR Master Mix (Roche, Mannheim, Germany) under standard thermal conditions (LightCycler[®] 480 Instrument II; Roche). The PCR reaction included 100 ng of cDNA, 10 \times concentrated PCR primers, 10 μ L of Master Mix, and RNase free water in a final volume of 20 μ L. The final concentration of primers was 1 mM. The qPCR conditions were: pre-incubation at 95°C for 5 min followed by 45 rounds of denaturation at 95°C for 10 s, annealing at 58°C for 20 s, extension at 72°C for 30 s, followed by dissociation curve analysis. The NormFinder software (MOMA, Aarhus, Denmark) was used to obtain a set of candidate normalization genes according to their expression stability in a given sample set and given experimental design. The expression levels of genes of interest were normalized to the housekeeping 18S ribosomal RNA gene (RNA18S1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and b-actin (ACTB) expression using the $\Delta\Delta$ Ct method to calculate the relative mRNA levels of each target gene for the entire MSCs population.

Statistical analysis. All results are expressed as mean \pm standard error. The significance of differences among the means of data for gene expression and the GAG quantity were analyzed using one-way analysis of variance and the Tukey test as a *post hoc* test. A p-value of less than 0.05 was considered statistically significant.

Results

Isolation, microencapsulation and 3D cultures of MSCs and its sub-populations for in vitro studies
In all three investigated cell populations, MSC phenotype was confirmed by immunocytochemistry.

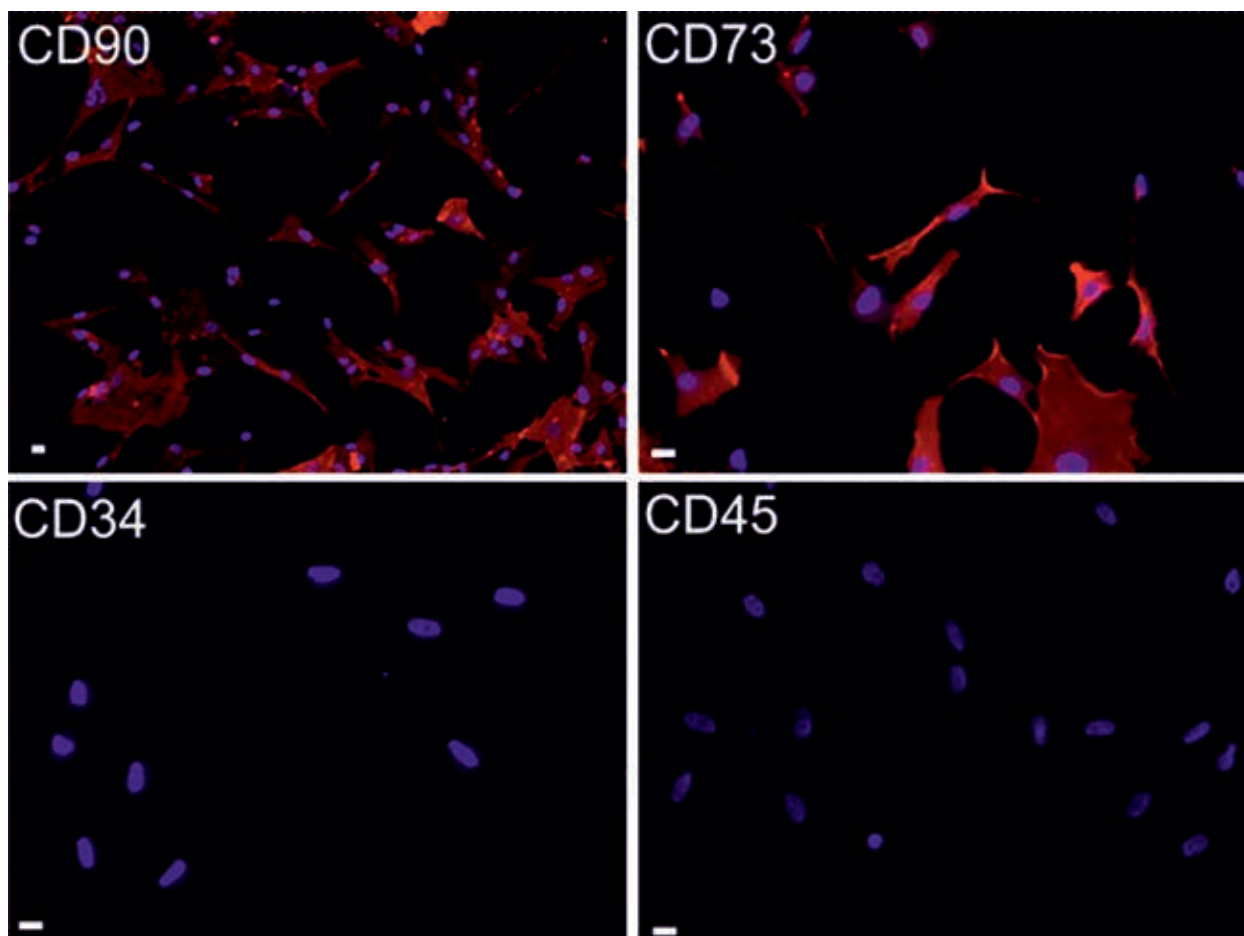


Figure 1. Expression of cell surface markers in the analyzed mesenchymal stem cells subsets. Presence of CD73 and CD90 proteins (red) and absence of CD45 and CD34 expression, Hoechst nuclear stain (blue). Scale bar: 10 μm .

The cells were immunopositive for CD90 and CD73 markers, and immunonegative for CD34 and CD45 markers (Fig. 1). Following cell count, cells were microencapsulated in collagen microspheres after reaching the plateau phase in about 2 weeks of culturing as monolayers. MSC microspheres contained 5×10^5 cells of each subset.

Glucosaminoglycans content in cell microspheres

To evaluate the differentiation process of MSCs, GAGs' content produced by cells in the microspheres was measured and expressed in $\mu\text{g}/10^5$ cells. Both MSC subsets showed higher GAG biosynthesis activity (1.97 $\mu\text{g}/10^5$ cells and 3.38 $\mu\text{g}/10^5$ cells for CD271⁺ MSCs and CD271⁻ MSCs, respectively) than the control PA-MSCs (0.73 $\mu\text{g}/10^5$ cells).

New GAG matrix was produced by both MSC subsets as shown by the increased GAG content; however, the significant difference was observed between CD271⁻ MSCs and PA-MSCs (Fig. 2).

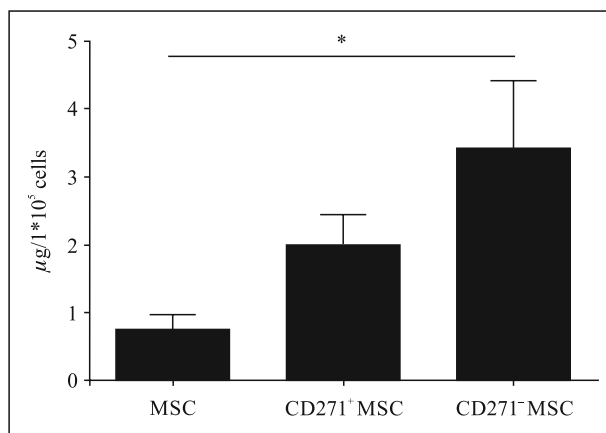


Figure 2. GAG content in the analyzed subsets of MSC evaluated by 1,9-dimethylmethylene blue (DMMB) dye-binding assay. *Statistically significant difference, $p < 0.05$.

Expression of NP gene markers by qPCR

The concentration and quality of the total RNA were estimated by measuring the absorbance

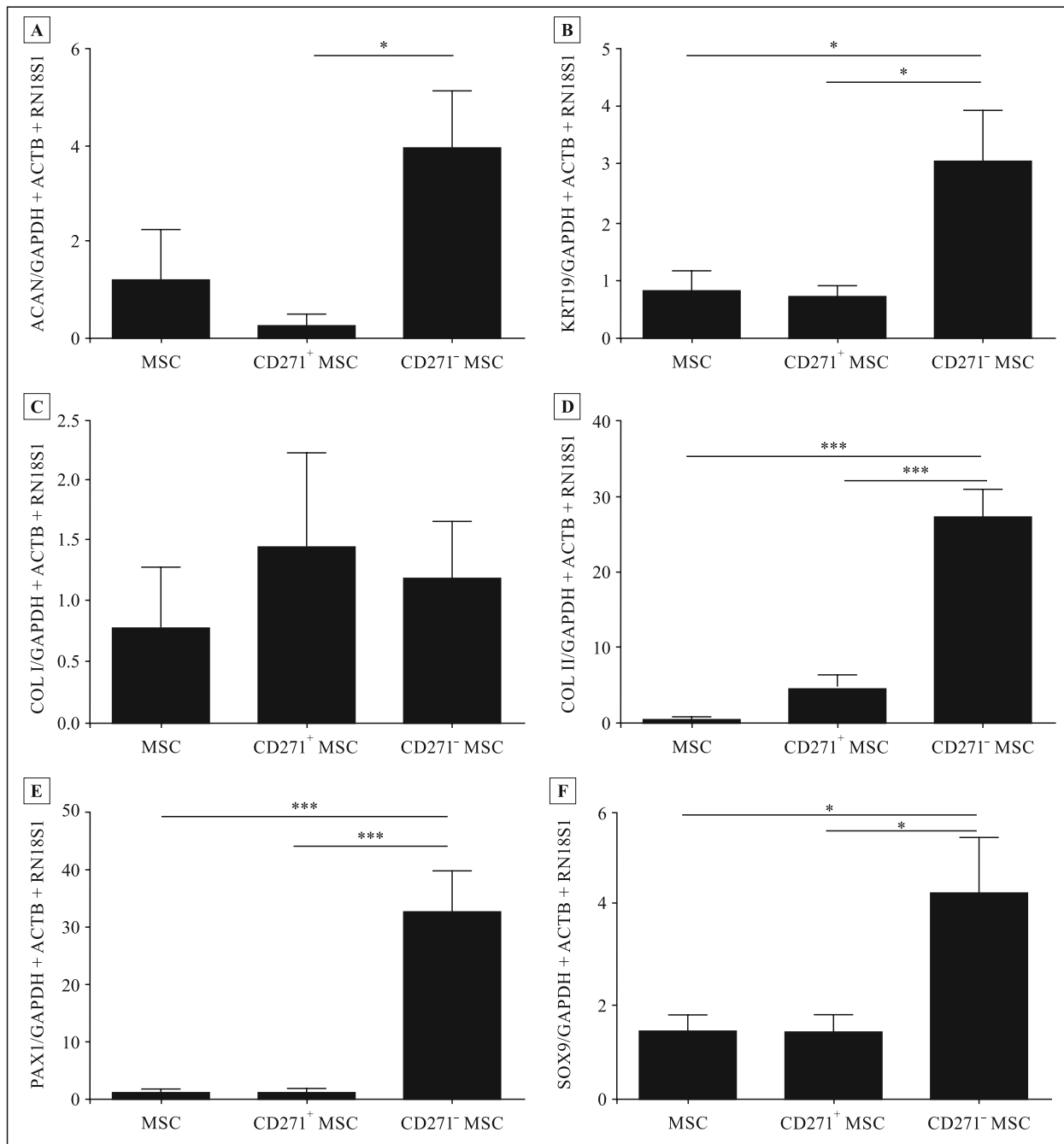


Figure 3. Gene expression in the analyzed subsets of MSC evaluated by real-time PCR. **A.** Aggrecan (ACAN); **B.** Keratin 19 (KRT19); **C.** Collagen type I (COL1A1); **D.** Collagen type II (COL2A19); **E.** PAX1; **F.** SOX9. *, ***Statistically significant differences, $p < 0.05$ and $p < 0.001$, respectively.

ratio of 260/280 nm and range 40–200 ng/ μ L and 1.80–1.99, respectively. To follow MSC differentiation process the expression of the nucleus pulposus gene markers was analyzed using quantitative PCR. Gene expression analysis of matrix proteins regarded as phenotypic markers of nucleus pulposus chondrocyte-like cells, showed markedly higher expression in CD271⁻ MSC of the all studied mark-

ers, with the exception of collagen type I (Fig. 3C). Expression of keratin 19 and collagen type II genes was significantly higher in CD271⁻ MSCs compared to CD271⁺ MSCs and PA-MSCs, 3 fold and 30 fold, respectively. The aggrecan gene expression was significantly higher (4-fold) in CD271⁻ MSCs than in CD271⁺ MSCs (Fig. 3A–C). Collagen type I mRNA levels were similar in all the studied cell populations (Fig. 3D).

We measured gene expression of transcription factors characteristic for normal intervertebral disc [36] and found that both PAX1 and SOX9 showed higher expression in CD271⁻ MSCs, as compared to the CD271⁻ MSCs and PA-MSCs, nearly 30-fold and 2-fold, respectively (Fig. 3E, F).

Discussion

The CD271 antigen has been identified as a marker of the most homogeneous mesenchymal stem cell (MSC) subset [36]. To date, CD271 molecule has been described as one of the most specific markers for the isolation of bone marrow BM-MSCs. It is now well documented that the proliferation and differentiation potential of CD271⁺ bone marrow-derived MSCs is higher than that of the unfractionated source mixture of the bone marrow-derived MSCs [36]. Since the CD271 antigen is able to promote cell proliferation and differentiation along three mesenchymal lineages, *i.e.* adipogenic, osteogenic and chondrogenic [27, 28, 38–40], it has been intensely investigated as an antigen driving generation of differentiated cells suitable for specific therapeutic applications. Obtaining the chondrocyte-like phenotype in an attempt to regenerate cartilage has been one of these experimental directions. Because it was found that the CD271⁺ MSCs can effectively differentiate towards cartilage tissue [15, 27, 28], we decided to check if these cells can differentiate into chondrocyte-like cells of the nucleus pulposus (NP) of IVD. However, since CD271 is a very efficient nerve growth factor receptor, its presence may lead to nerve ingrowth into NP. Therefore, we also examined phenotype of CD271⁻ MSCs and used PA-MSCs as a control cell population for the both isolated CD271 cell subsets. Although the phenotype of NP cells is closely associated with that of chondrocytes, still clear differences exist [41]. Similarly to the *in vitro* differentiation of MSCs into chondrocytes, their differentiation toward an NP-cell phenotype depends on diverse and specific parameters such as an appropriate choice of growth factors, 3D-matrix, intercellular contacts or environmental conditions mimicking the physiological status of IVD such as hypoxia [34].

It is generally accepted to use phenotypic markers such as aggrecan and collagen type II, characteristic of articular cartilage, to define the nucleus pulposus cells phenotype [42–44].

Genes encoding molecules characteristic for the NP may be valuable for monitoring expression changes during differentiation of MSC subsets toward chondrocyte-like cells of nucleus pulposus. With respect to the typical chondrogenic markers, aggrecan, collagen

type II, keratin 19, Sox9, PAX1 and glycosaminoglycans, the use of different populations of MSCs in our study demonstrated clear differences in chondrogenic gene up-regulation and accumulation of GAGs.

A healthy intervertebral disc is highly hydrated, the water content being a key parameter responsible for the biophysical properties of the NP [45]. Abundance and unique molecular features of GAGs and aggrecan provide IVD with its osmotic properties and ability to withstand compressive loads, whereas their degradation results first in the impairment of disc function and then the onset of its degeneration [46]. IVD degeneration is associated with changes in disc morphology and composition of extracellular matrix, which lead to the loss of correct shape and water content. Therefore, to achieve optimal disk recovery, an increase in the synthesis of the water-absorbent particles needed to restore the cushioning actions is needed [47]. We have indeed observed a significant increase in the GAG biosynthesis and in aggrecan expression in the CD271⁻ MSCs, but not in CD271⁺ MSC and not in PA-MSC populations (Fig. 2, 3A). The results obtained by others for these two macromolecules differ between reports and largely depend on the BM-MSC growth conditions [30, 48].

SOX9, responsible for the induction of type II collagen expression later during chondrogenic differentiation, is the most widely used marker in the evaluation of MSC differentiation to chondrocyte-like cells [49]. We have found that CD271⁻ MSCs showed a significant increase in collagen II and SOX9 expression compared to the CD271⁺ MSCs and PA-MSCs. Others have shown an increase in type I collagen and a reduction in type II collagen present in the degenerating NP [50]. There was no difference in the COL I expression between any of the experimental groups (Fig. 3C). Keratin 19 is another important marker of healthy discs, also known as a NP marker in rat and human [42, 51, 52]. Keratin 19 is frequently described as a notochordal cell marker [51], expression of which is induced by the low oxygen tension [53]. Parallel to the results obtained for other NP markers, we observed a significant increase in the expression of keratin 19 in the CD271⁻ MSCs subset (Fig. 3B). Others have reported significant keratin 19 expression in BM-MSC [33]; however, because of multiple cell types present in BM-MSC and different culture conditions used, it is impossible to discuss the expression of any markers. Additionally, there may be yet unknown interactions between various cellular BM-MSC components, that may influence each other and cross-regulate biology of subsets co-existing in one MSC source.

Nerve growth factor (NGF) is a key neurotrophic factor that promotes ingrowth and survival of nerves

[54, 55]. Degeneration-initiated changes associated with rearrangements of innervation, are linked to the increase of nerve ingrowth in the NP, which leads to the chronic back pain [56]. Absence of CD271, a NGF receptor, should therefore be a favorable feature of cell-based NP-regenerative therapy.

Here we have demonstrated, for the first time, a great capacity of isolated CD271⁻ MSCs to produce extracellular matrix components necessary for supporting function of healthy IVD. We have shown that: (i) CD271⁻ MSCs present a higher differentiation potential toward chondrocyte-like cells of nucleus pulposus when compared to CD271⁺ MSCs; and (ii) the absence of CD271 in a subset of MSCs likely triggers a significantly higher differentiative potential toward nucleus pulposus in these cells than in CD271⁺ MSCs.

Overall, presented results show that CD271⁻ MSCs may be superior candidates for NP restorative treatment compared to CD271⁺ MSCs and PA-MSCs.

Acknowledgements

This work was sponsored by National Science Center (NCN) grants number 2012/07/B/NZ4/01427 and 2012/05/D/NZ3/02028.

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Submitted: 19 May, 2017

Accepted after reviews: 14 August, 2017

Available as AoP: 18 August, 2017