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• LETTER TO THE EDITOR •

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Isolation and characterization of mesenchymal stem-like cells from human nucleus pulposus tissue

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Dear Editor,

Large numbers of individuals experience low back pain (LBP) during their lifetime [1,2]. LBP excruciates approximately 80% aging population and causes significant socio-economic problem [3,4]. LBP often originates from the intervertebral disc degeneration (IVDD). The intervertebral disc (IVD) is a specialized biomechanical complex composed of a hyper-hydrated and oligocellular central structure, namely, the nucleus pulposus (NP), and the annulus fibrous (AF) [5]. Most current available treatments for IVDD only work on symptomatic relief, and do not cure this disease. MSCs could be a cell therapy candidate in clinical IVD regenerative therapies due to the avascular characteristic of NP [6]. MSCs are distributed in all tissues and recognized by their ability to self-renew, proliferate, and differentiate into several cell types. In the last few years, Blanco et al. [7] identified MSCs from the degenerate human NP and compared them with bone marrow (BM) MSCs. They concluded that MSCs obtained from NP are quite similar to BM-MSCs, with the exception of their adipogenic differentiation ability.

In this study, with the patients' informed consent, seven disc samples were obtained from patients who underwent various spinal procedures (Table S1 in Supporting Information). The age of the donors ranged from 10 to 37. The NP tissues were carefully separated from the AF using surgical loupes without any visible contamination by the AF or any other tissue. NP tissue was mechanically fragmented and subjected to enzymatic digestion with collagenase II and trypsin. The digests were plated in a chemically defined medium, DMEM/F12. Cultures were passaged when they reached 80% confluence.

In all cases, MSC-like cells from seven human NP tissue samples were expanded successfully. The cells cultured in plates presented polygonal-shape in the first generation, and mainly spindle-shape when passaged to the fifth generation (Figure 1A). MSC-like cells isolated from NP were spindle-shape, which were similar to BM-MSCs derived from bone marrow.

In order to further characterize the cells isolated from the NP tissues, the flow cytometric immunophenotyping was performed to all seven samples**.** Cell surface markers of the fifth generation cells were examined by flow cytometric analysis (Figure S1 in Supporting Information). Compared with the isotype control IgG1, about all cells were positive for CD44, CD29 and CD105 and negative for CD34, CD45, CD14 and HLA-DR, indicating that they are MSC-like cells. The result showed that MSC-like cells were isolated from human NP-tissues and expanded successfully.

The cells presented similar growing behaviors (such as morphology) during passages and characteristics analyzed by flow cytometric immunophenotyping for all the seven NP samples. But the cell morphology of the passage 2 (polygonal-shape) differs with that of the passage 5 (spindle-shape) evidently. The cells at passage 2 and 5 from the

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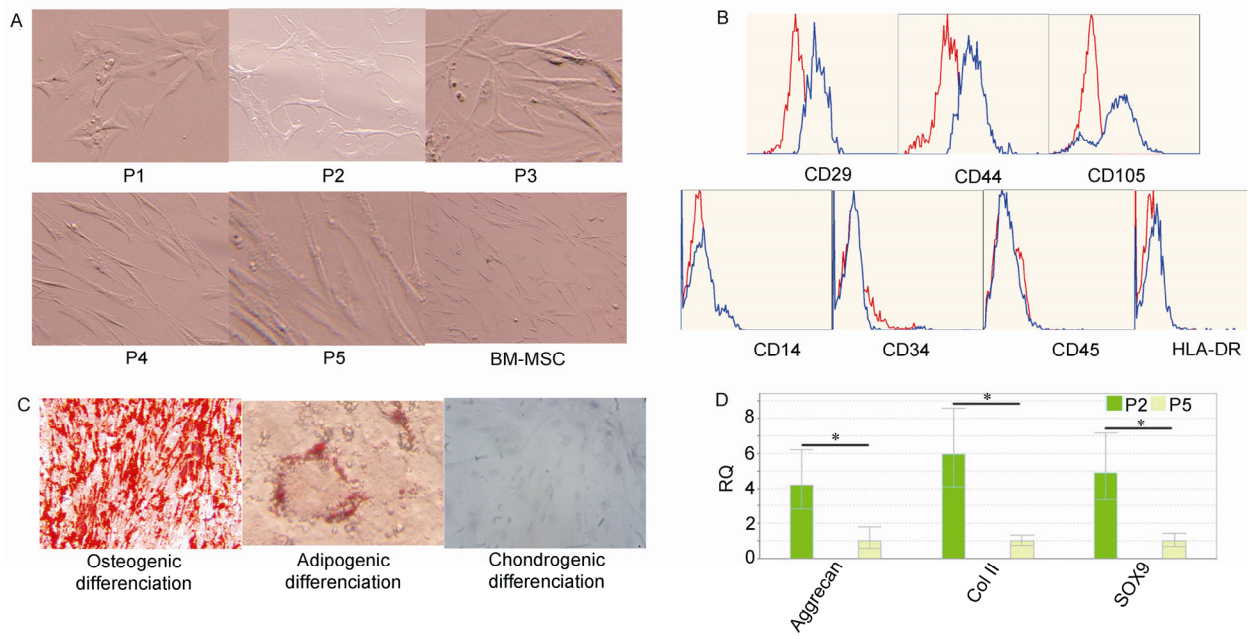

Figure 1 Characteristics of MSC-like cells from NP tissue and comparison of MSC-like cells between P2 and P5. **A**, MSC-like cells isolated from NP were expanded *in vitro* from passage 1 to 5. The BM-MSC (bone marrow MSC) is as control. The MSC-like cells were spindle-shape similar to that of BM-MSCs. B, Immunophenotypic histograms of P2 (red) and P5 (blue) cells, analyzed by FACS, were positive for CD44, CD29 and CD105 and negative for CD45, CD34, CD14 and HLA-DR. C, Multilineage differentiation of MSC-like cells. D, The mRNA quantification of the NP extracellular matrix markers in P2 and P5 cells were analyzed by real-time PCR. The results of each experiment are expressed relative to the quantity of β-actin mRNA transcription as an internal control.

patient No.1 were collected and further analyzed as representation by real-time PCR and immunophenotyping comparison. Fluorescence intensity comparison between P2 and P5 MSC-like cells was performed by flow cytometric analysis. The fluorescence peaks of the makers CD44, CD29 and CD105 of the passage 5, with higher intensity than that of P2, separated away from that of the passage 2; whereas the fluorescence peaks of the makers CD34, CD45, CD14 and HLA-DR of the passage 5 and the passage 2 were nearly overlapped (Figure 1B). The results indicate that the concentration of MSC-like cells of the passage 5 is higher than that of the passage 2, suggesting that the MSC-like cells are truly expanded with the passage. In addition, the mRNA levels of NP extracellular matrix specific markers, including *collagen type II*, *aggrecan*, and *SOX9*, were quantified by real-time quantitative PCR. The mRNA level of all three markers in P2 cells was dramatically higher than that in the P5 cells ($P<0.05$ for each) (Figure 1D). This assay suggests that the quantity of NP cells are larger in P2 than that in P5, and they do not get expanded with the passage due to the proliferation capability of the NP cell is weaker than that of MSC. Combination of the immunophenotyping analysis and real-time PCR results suggested that the MSC-like cell population of P5 was larger and more homogeneous than that of the P2, and indicated that the MSC-like cells were proliferated along with the generations.

The expanded cells obtained from the six among the seven NP samples could differentiate into osteocytes, adipocytes, and chondrocytes (Figure 1C), but only the NP samples from the patient No. 7 could not differentiate into adipocytes. This result is in accordance with the report by Blanco et al. that MSCs from IVD are able to differentiate into both osteocytes and chondrocytes, but cannot differentiate into adipocytes in any of the cases [7]. The age of the patients in the case may account for the difference. MSC prefers to differentiate into osteogenesis cells, rather than adipogenesis cells along with the age [8]. The average age of the patient 1–6 is 18.5 years old, and the seventh patient is 37 years old, the only one who's MSC-cells cannot be adipogenic differentiation. The average age in cases reported by Blanco et al. is older than that in our cases, which could explain that there were no adipogenesis cells in their differentiation assays.

In short, this report demonstrated that NP tissue contains MSC-like cells which could be isolated and proliferated *in vitro*. The spindle shape may be referred to as a morphological characteristic of the MSC-like cells, although it needs more verification. The morphological, immunophenotypical and differentiation characteristics of the MSC cells proliferated met the criteria described according to the International Society for Cellular Therapy (ISCT) for MSC. Recent research focused on tissue engineering and induced MSC engineering for treating IVDD. Isolation of MSC-like cells from NP tissues and proliferation *in vitro* provide a platform for developing the therapies of IVDD with endogenous MSCs.

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Supporting Information

Figure S1 Immunophenotypic histograms of MSC-like cells analyzed by FACS, IgG1 was the isotype control.

Table S1 Patient Age, Sex, Surgical Indication, Level of Disc Specimen, and procedure

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