Differentiation of Mesenchymal Stem Cells into Nucleus Pulposus-Like Cells Induced by Co-Culture System and Hypoxia

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Abstract

Introduction: Intervertebral disc (IVD) is closely related to low back pain, is the major cause of disability worldwide, with more than 84% population experiencing pain in their life time. Biological and cell based therapies are on progress as an optional treatment for IVD degeneration. Many studies have also revealed that mesenchymal stem cells (MSCs) can also be differentiated into nucleus pulposus (NP) like cells phenotype. This study aimed to determine the newly defined healthy NP cells markers; HIF-1α, HIF-2α, GLUT-1, Shh, Brachyury, Aggrecan, Collagen II, Carbonic anhydrase 3, Carbonic anhydrase 12, CD24, Cytokeratin 8, Cytokeratin 18, and Cytokeratin 19 of Sprague-Dawley rat, whether these markers can be expressed in MSCs under co-culture condition and could be identified the differentiation of MSCs into NP-like cells.

Methods: NP cells and bone marrow derived MSCs from Sprague-Dawley rats were cultured under normoxic medium at 21% O_2 and 5% CO_2 at 37˚C and hypoxic medium at 2% O_2, 5% CO_2, 93% N_2 at 37˚C and MSCs were co-cultured with NP cells supernatant with the concentration of 50% and 100% for 7 days under both normoxic and hypoxic medium. Differentiation of MSCs and expression of recommended newly defined young healthy NP cells phenotypes were evaluated by quantitative real-time PCR (qPCR), western blotting and immunofluorescence staining microscopy. The results were determined among the groups using unpaired Student's t-test. p-values <0.05 considered significant.

Results: MSCs co-cultured with the concentration of 50% NPcs supernatant; only collagen II showed the increased expression while with the 100% NPcs supernatant; brachyury, collagen II, Glut-1, KRT18 and KRT19 showed higher expressions under normoxic condition compared to MSC control. Under the hypoxic condition, MSCs co-cultured with 50% NPcs supernatant, HIF-2α, Glut-1, aggrecan, collagen II, shh, KRT8, KRT19, CA3, CA12 and CD24 showed increased expression compared to MSC control. More importantly, MSCs co-cultured with 100% NPcs supernatant under hypoxic condition, HIF-1α, HIF-2α, Glut-1, aggrecan, collagen II, shh, brachyury, KRT8, KRT19, CA3, CA12 and CD24 showed upregulated increased expressions compared to the MSC control, which showed that NP cells can stimulate MSCs differentiation to NP-like cells with paracrine interaction between MSCs and NPcs under co-culture condition.

Conclusion: This study suggested that MSCs were successfully differentiated into NP-like cells, which may be used as an ultimate cell-based therapy for IVD regeneration.

Keywords: *Co-culture, Cell Phenotype, Nucleus Pulposus Cell, Mesenchymal Stem Cell*

Images:

Fig. 1

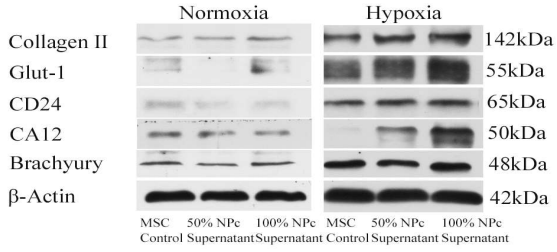


Fig.2

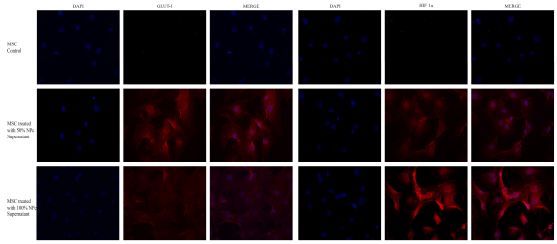


Fig. 1: Relative protein expression by western blot in MSCs co-cultured with NPcs supernatant (50% and 100% concentration) under normoxia and hypoxia conditions for 7 days. Protein expression for each samples were normalized with housekeeping gene β-actin.
Fig. 2: Relative protein expression by immunofluorescence staining in MSCs co-cultured with NPcs supernatant (50% and 100% concentration) under hypoxia conditions for 7 days.

Professional Biography (100-150 words)

ARJUN SINKEMANI, male, 34 years old, has completed master degree in Orthopaedic Surgery from Southeast University and now pursuing his PhD study in the same university. He has more than 5 scientific publications. He has participated in the 20th Asia Pacific Orthopaedic Association Congress, Turkey- Oral Presentation; the 12th International Congress of Chinese Orthopaedic Association, China- E-Poster Presentation; AOSpine Advanced Symposium, China- Controversial Case Discussion Forum; Orthopaedic Research Society 47th International Musculoskeletal Biology Workshop, USA- Poster Presentation; International Symposium on Life Science & Biological Engineering, Hong Kong- Oral Presentation; International Symposium on Life Science & Biological Engineering, Japan- Oral Presentation and the 10th International Congress of Chinese Orthopaedic Association, China. He holds the active memberships of AOspine, Orthopaedic Research Society and North American Spine Society. He is awarded with the Chinese Government Scholarship for Outstanding International Students; Nanjing Municipal Government International Students Scholarship; 1st Category Southeast University Scholarship and Chinese Government Scholarship.



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