

Isolation and Characterization of Cell Subpopulation with Stem Cell Properties in Human and Monkey Intervertebral Disc (IVD)

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INTRODUCTION: Intervertebral disc (IVD) degeneration results in major public health problems including low back pain. Degeneration of IVD is an aberrant cell-mediated process involving significant tissue remodelling. While transplantation of exogenous stem cells has been proposed to be a promising therapy, induction or maintenance of endogenous regenerative properties may also provide an alternative or complementary approach. This ongoing study therefore aims at identifying, isolating and functionally characterizing the cells within the IVD that exhibit properties of adult stem cells.

MATERIALS & METHODS: Under the ethical approval from the local authorities, BrdU was intraperitoneally injected to mice and rats and DNA-retaining cells within their IVD were detected using immunohistochemical staining. IVD tissues were further obtained from health Rhesus Monkeys aged at 2~3 years and surgically operated patients with healthy IVD. Bulk cells were isolated from annulus fibrosus (AF) and nucleus pulposus (NP) tissues, respectively, by collagenase digestion of the tissue pieces. A subgroup of cells negative for Ochst33342 red and blue, i.e. the side population (SP), of AF and NP cells were detected. The isolated cells were also examined by flow cytometry for their expression of known stem cell markers such as CD9, CD271 and CD146. Cells were positively selected using Magnetic Affinity Sorting (MAS) based on the expression of these markers. The clonogenicity of the isolated cells and differentiation potentials were assessed using standard protocols. In vivo self-renewal of selected cells were further tested in nude mice after transplanted.

RESULTS: BrdU-labelling retaining cells in mouse and rat IVD could be detected in as long as 14-16 weeks after BrdU injection. In monkey and human samples, NP cells were demonstrated to express CD9 and CD271 and AF cells expressed CD9, CD271 and CD146. CD146⁺ AF cells showed a clonogenic potential higher than other subgroup cells (Fig. 1). In vitro differentiation assays on CD146⁺ AF cells also

showed stronger lineage multipotent capacity while both AF and NP cell populations could differentiate towards osteogenic, adipogenic and chondrogenic lineage cells (Fig. 2).

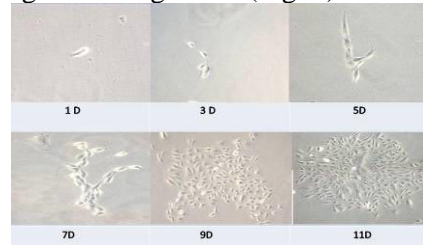


Fig. 1. Colony forming from CD146⁺ cells isolated from Rhesus monkey.

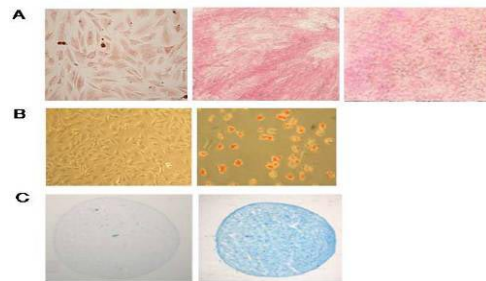


Fig. 2. Differentiation of CD146⁺ AF cells towards 3 mesoderm lineage cell types. A. Osteogenic lineage cells stained for Alizarin red and ALP, respectively. B. Adipogenic lineage staining for Oil red O(C). C. Chondrogenic lineage stained for Alcian blue.

DISCUSSION & ONGOING WORK: Our work has shown that both the NP and AF portions of the IVD contain cell populations with stem cell properties such as clonogenicity and multiple differentiation potentials, which support a recent published report [1]. Interestingly, cells from AF seem to express the higher level of CD146, a broadly accepted marker for mesenchymal stem cells [2], and to display higher differentiation potentials. Further work in comparison with regenerative properties of bone marrow-derived mesenchymal stem cells is ongoing. Furthermore, the maintenance of self-renewal properties of IVD stem cells is also being tested after transplanted in nude mice.

REFERENCES: ^[1]MV. Risbud et al., Spine, 2007, 32 2537-2544. ^[2]A. Sorrentino et al., Experimental Hematology. 2008, 36 1035-1046.