

Molecular Phenotypes of Notochordal Cells Purified from Nucleus Pulposus via Fluorescence-Activated Cell Sorting

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INTRODUCTION: The immature nucleus pulposus (NP) is populated by cells of notochordal-origin that are larger and contain more extensive cytoskeleton and vacuoles [1,2,4]. The disappearance of these cells with age is believed important in regulating metabolic shifts that occur in the aging intervertebral disc [1]. Work in our laboratory has shown that cells derived from the notochordal cell-containing NP do not respond to physical stimuli and soluble mediators to the same extent as fibrochondrocyte-like cells of the disc [3], suggesting a unique phenotype for these cells. In this study, we developed a new technique to purify notochordal-like cells from immature NP cells by fluorescence-activated cell sorting (FACS) and characterized their unique molecular phenotype by mRNA and integrin expression patterns.

METHODS: Primary cells were isolated from the annulus fibrosus (AF) and NP of skeletally-immature porcine and rat spines. NP cells were sorted by both fluorescence and size on the FACStarPLUS with smaller AF cells (10-15 μ m cell size) as the reference population. Sorted cells were collected into two fractions: large NP cells (higher fluorescence and larger than AF cells) and small NP cells (lower fluorescence and smaller than AF cells). Real-time RT-PCR analysis was used to characterize gene expression in these two cell populations for key extracellular matrix related proteins (collagen types I and II, aggrecan, decorin, biglycan, lumican, MMP1, 2 and 3, TIMP1 and 2). Expression levels of integrin subunits (α 1, α 5, α 6, β 1) were analyzed by flow cytometry with appropriate antibodies.

RESULTS: FACS analysis showed that the NP contained a majority of cells that were larger than AF cells (Fig. 1), with auto- and blue fluorescence higher than AF cells (Fig. 2). Microscopic examination of the sorted large NP cells demonstrated the existence of vacuoles within many cells of this population, consistent with the appearance of notochordal cells identified in previous reports [1,2,4]. In comparison to the sorted small NP cells, large NP cells expressed lower mRNA levels of type

I collagen, biglycan and TIMP1. A greater number of these large NP cells also expressed α 6 and α 1 integrin subunits and a higher expression level of β 1 subunits as compared to small NP cells (Table). These differences point towards a potential difference in integrin-mediated interactions with collagens and laminin in the matrix of NP.

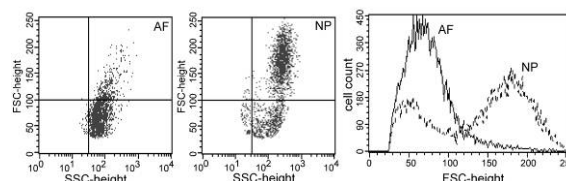


FIGURE 1: Flow cytometry analysis by light scatter only for cell size in rat IVD cells.

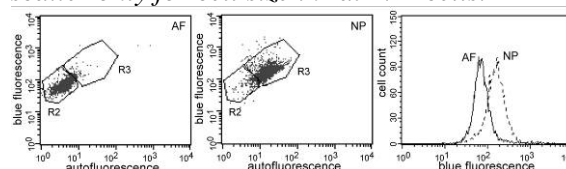


FIGURE 2: Flow cytometry analysis by auto and blue fluorescence in rat IVD cells

TABLE. % positive cells and mean fluorescence intensity (MFI) of integrins in porcine NP cells.

subunit	Large NP		Small NP	
	% of (+) cells	MFI	% of (+) cells	MFI
α 1	35	10	10	5
α 5	74	27	55	19
α 6	39	20	19	9
β 1	96	124	95	67

CONCLUSIONS: Notochordal-like cells of the NP have a molecular phenotype (i.e. mRNA and integrin expression) distinct from that of the small NP cells, suggesting their unique metabolic contributions and interactions in the intervertebral disc. This technique presents the possibility to identify unique gene expression profiles or metabolic markers for these notochordal-cell like population.

REFERENCES: ¹Aguilar DJ et al.(1999) *Exp Cell Res* 246,129. ²Guilak F et al.(1999) *Spine* 24,2475. ³Setton LA &Chen J(2004) *Spine* 29, 2710. ⁴Trout JJ et al.(1982) *Anat Rec* 204,307.

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