CELLULAR, BUT NOT MATRIX, IMMUNOLOCALIZATION OF SPARC IN THE HUMAN INTERVERTEBRAL DISC: DECREASING LOCALIZATION WITH AGING AND DISC DEGENERATION

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INTRODUCTION: Although many investigators recognize SPARC (secreted protein, acidic, and rich in cysteine) as a major protein of mineralized bone, SPARC plays another important role as an extracellular matrix glycoprotein modulating the interaction between cells and their extracellular matrix (ECM). Because of this important function in ECM remodeling, the present study was undertaken to investigate the presence of SPARC in the human intervertebral disc. Although one previous study reported no immunolocalization of SPARC in either fetal or adult disc tissue [1], the present study showed positive immunolocalization in disc cells and showed decreasing localization during aging and disc degeneration.

METHODS: Experimental studies were approved prospectively by the authors’ Human Subjects Institutional Review Board. Patient specimens were derived from surgical disc procedures performed on individuals with herniated discs and degenerative disc disease. Surgical specimens were transported to the laboratory (less than 30 minutes after surgical removal) in sterile tissue culture medium and placed in 10% neutral buffered formalin. Care was taken to remove all granulation tissue and to sample only disc tissue. Control specimens were obtained via the National Cancer Institute Cooperative Human Tissue Network; they were shipped overnight to the laboratory in sterile tissue culture medium and placed in 10% neutral buffered formalin. Specimens were embedded in paraffin. Tissues were examined from 8 young control donors aged newborn to 10 years and 11 surgical disc specimens from patients aged 15-76 years. Surgical and control specimens had the following Thompson scores: grade II, one specimen; grade III, 4 specimens; grade IV, 5 specimens.

SPARC immunolocalization: Disc specimens were embedded in paraffin and sectioned, deparaffinized and hydrated to distilled water. Sections were incubated with proteinase K (Sigma, St. Louis, MO), 20 µg/ml, for 15 min. at room temperature (RT), and rinsed. Endogenous peroxidase was removed by incubation in 3% H2O2 (Sigma in methanol for 5 min. at RT). Sections were rinsed with distilled water and with 2 changes of 1% phosphate buffered saline (PBS) (Boehringer-Manheim), and incubated with 10% normal horse serum in base solution (consisting of 4% bovine serum albumin, and 5% non-fat dried milk in 1% PBS) for 10 min. at RT. Primary antibody was mouse anti-SPARC (Developmental Studies Hybridoma Bank) with a biotinylated horse anti-mouse secondary antibody (Vector Labs). Horse serum was left on sections to be used as negative controls. Cell Culture Methods: Primary cultures were grown as previously described [2]. Following primary culture, cells were subsequently passaged in either monolayer culture (seeded at 5,000 cells/well of a Lab-Tek® Chamber SlideTM (Nunc, Napierville, IL) or into 3D culture in agarose at a cell density of 30,000 cells. The agarose was then layered onto a Costar Transwell Clear Insert (Costar, Cambridge, MA). Cells were cultured for 5 days in monolayer or 10 days in agarose. Cells were grown as previously described [1] in sterile modified Minimal Essential Medium with Earle’s salts, non-essential amino acids, penicillin-streptomycin and 20% fetal bovine serum.

RESULTS: SPARC was localized in the disc cell cytoplasm but not in the ECM of any of the specimens. In discs from young newborn donors, SPARC was localized in all cells of the outer annulus, and the majority of cells in the inner annulus; in the nucleus, however, many cells showed no localization. [Figure 1A-C]. By age 4.7 years, however, localization was positive in only some annulus cells. Specimens of tissue from degenerating discs also showed variable cellular localization [Figure 2A-D]. Disc cells cultured in 3D in agarose [Figure 3A and B] and cells cultured in monolayer [Figure 4] showed positive localization in all cells.

DISCUSSION: This study is important because it demonstrates the first documentation of the presence of SPARC in the human intervertebral disc and shows decreased presence of SPARC with aging disc structurally to the ECM but rather may act to regulate production of several ECM proteins [3]. Based on findings in other tissues [4,5,6], immunodetection of SPARC in the present study suggests that SPARC may have heretofore unrecognized functions in the disc which influence collagen fibrillogenesis, deposition and remodeling, and may also modulate growth factor efficacy. Novel findings presented here point to the importance of future studies to elucidate the role of SPARC in disc aging and degeneration, ECM remodeling, metalloproteinase expression and growth factor efficacy.

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Figure 1. Immunolocalization of SPARC in disc tissue from a newborn: A, outer annulus; B, inner annulus, C, nucleus; D, negative control.  
Figure 2. Immuno-localization in annulus tissue from a 4.7 year old (A), 10 year old (B), 15 year old (C) and 76 year old (D). Arrows point to cells negative for immunolocalization of SPARC.

Figure 3. Immunolocalization in cells from the annulus cultured for 10 days in agarose. Localization is positive in both small (A) and large (B) cell groupings. C, negative control.  
Figure 4. Immunolocalization in cells from the annulus cultured for 5 days in monolayer.

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