Ultrastructural & Analytical Methods in Life Sciences

LS.6.P159
Immunofluorescence distribution of cytoskeletal and extracellular matrix antigens in dogfish Scyliorhinus canicula L. notochord

I. Dujmovic[1], I. Bocina[2], K. Vukojevic[2,3], M. Saraga-Babic[2,3]

[1]Faculty of Philosophy, Education department, Split, Croatia
[2]Faculty of Science, University of Split, Split, Croatia
[3]School of Medicine, University of Split, of histology and anatomy, Split, Croatia

idujmovic@ffst.hr
Keywords: dogfish, *Scyliorhinus canicula*, notochord, cytoskelet, extracellular matrix

Notochord is a structure that defines all members of the phylum Chordata, and plays an important role in the vertebrate development. It occurs during early embryogenesis; it is main skeletal element of embryo and serves as a source for the formation of the surrounding tissue [1]. Dogfish *Scyliorhinus canicula* L., is a cartilaginous fish, evolutionary positioned among cephalochordates (*Cephalochordata)* and teleost fish (*Osteychtyes*), and thus very interesting as an object of research [2].

In most species, notochord cells are vacuolated and becoming bigger in time, producing a thick three-layer sheath composed of collagen fibers and glycosaminoglycans [3-5]. Vacuolated cells permits elongation of growing embryo, as well as amortization and accommodations on biomechanical forces to the spine in the mature stage. Notochord is the first embryonic cellular structure that actively produces fibrillar extracellular matrix [6].

In this study we examined the immunohistochemical composition of notochord in ten young, both sexes specimens of dogfish *Scyliorhinus canicula* L., caught in the Adriatic Sea. The tissues were sectioned at cervical, truncal and caudal parts. Immunofluorescence techniques were applied on paraffin sections and observed under fluorescence microscope (Olympus BX61, Tokyo, Japan). We applied the antibodies against hyaluronic acid, citokeratin 8 and vimentin.

Applied immunofluorescence techniques have shown presence of cytoskeletal and extracellurar components in dogfish notochord and surrounding tissue. Hyaluronic acid positive cells of different intensity were seen in several parts of dogfish vertebra, including neural and hemal arch, as shown in “Figure 1a.”. Positive cells on hyaluronic acid were observed in the centrum of vertebra and in some notochordal cells ("Figure 1b.“). Since, in adult vertebrates the notochord retains only in nucleus pulposus of the intervertebral disc, whose main characteristic is proteoglycan-rich extracellular matrix, the presence of glycosaminoglycan hyaluronic acid in dogfish indicates the notochordal origin of nucleus pulposus cells. Double immunofluorescent staining to citokeratin 8 and vimentin confirmed the presence of these intermediate filaments in notochordal cells and vertebral centrum ("Figure 1c.“), indicating dogfish cartilaginous vertebra. Applied antibodies on sections through the cervical vertebra have shown that some notochordal cells coexpressed citokeratin 8 and vimentin, while some of them were labeled only by vimentin antibody ("Figure 1d.“).

The presence of similar cytoskeletal and extracellular matrix components confirmed in dogfish as well as in other vertebrates indicates the preservation of genes and proteins during evolution.

1. Stemple, D.L., Development 132(11), (2005). p. 2503-12.
2. Coolen, M., et al., CSH protocols (2008) p. pdb.emo111.
3. Adams, D.S., R. Keller, and M.A.R. Koehl, Development 110(1), (1990). p. 115-130.
4. Bocina, I. and M. Saraga-Babic, International journal of biological sciences 2(2), (2006), p. 73-8.
5. Risbud, M.V., T. Schaer, and I.M. Shapiro Developmental Dynamics 239(8), (2010). p. 2141-2148.
6. Risbud, M.V. and I.M. Shapiro, Critical Reviews in Eukaryotic Gene Expression 21(1), (2011). p. 29-41.

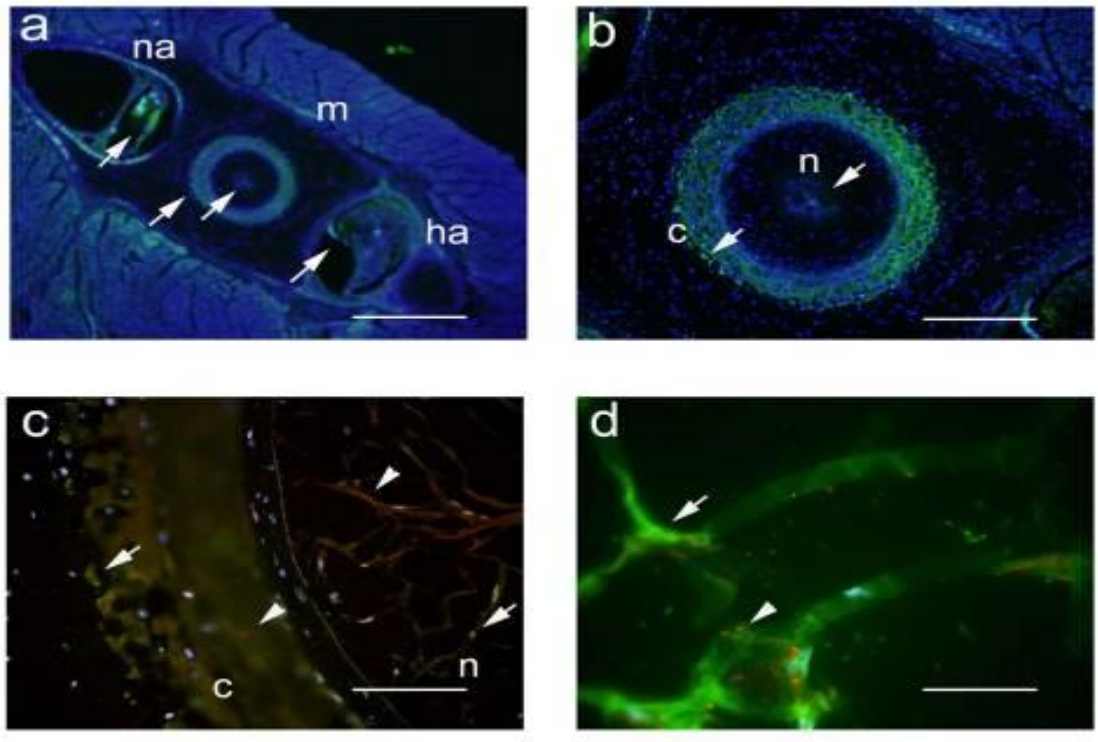

Figure 1. Transverse sections throughout dogfish *Scyliorhinus canicula* L. vertebra. a - Hyaluronic acid positive cells (*arrow*) of different intensity are seen in neural arch (na), hemal arch (ha), centrum (c) and notochord (n) of vertebra, x4. *Scale bar* 250 µm. b - Positive cells on hyaluronic acid observed in the centrum (c) and in several notochordal cells (n), x10. *Scale bar* 100 µm. c – Immunostaining to vimentin (arrow) and citokeratin 8 (arrowhead) confirming presence of these intermediate filaments in vertebral centrum (c) and notochordal cells (n), x40. *Scale bar* 25 µm. d - Section through the cervical vertebra showing some notochordal cells coexpressing citokeratin 8 and vimentin (arrowhead), while some of them are labeled only by vimentin antibody (arrow), x100. *Scale bar* 10 µm.