IVD Formation in *Monodelphis domestica*

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PRECS Phenotypic Plasticity Research Experience for Community College Students

Introduction

Intervertebral discs (IVDs) are made up of three main parts, the nucleus pulposus (NP), the annulus fibrosus (AF), and the end plates.[1] In this project we focused on the nucleus pulposus and characterizing its formation. In mice it has been shown that the nucleus pulposus is completely derived from the notochord, an embryonic structure found in chordates.[1,] Cells from the notochord in the nucleus pulposus have been hypothesized to play a role in disc maintainance.[1]

One study found macroscopic disc degeneration in people rises from 16% at age 20 to 98% at age 70 based on autopsies.[2] This suggests that disc degeneration is a nearly universal plight of the aging human condition. Research into the disc formation may give insight into potential treatments for disc degeneration.

Mice and rats are a long established model in biomedical research. However, they are not always the best model, shown by studies into thalidomide, a drug tested on mice that proved to be harmful for human fetuses.[3] Not having an appropriate model can hinder research and lead to negative effects for the populace.[3]

Monodelphis domestica, the gray short-tailed opossum, a relatively small and easy to keep laboratory animal, is perhaps an appropriate model system in many cases.[4] One case in which we believe it would be a appropriate model system, because of its biology, is research into disc formation and degeneration. We believe this because the pups are born in a premature state and completion of posterior IVD formation is thought to be delayed until after birth.[4,5]

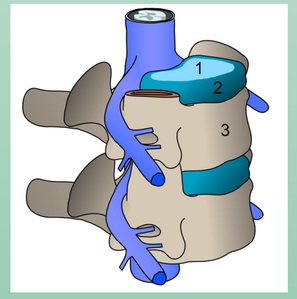

Figure 1: *Vertebral column.*
1: Nucleus pulposus.
2: Annulus fibrosus.
3: Vertebral body.
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Numbers added, image cropped.

Figure 2: *Left: Adult Monodelphis domestica. Right: 8 day old pup prepared as described in Figure 6 with head removed.*
Photo: Sears Lab.

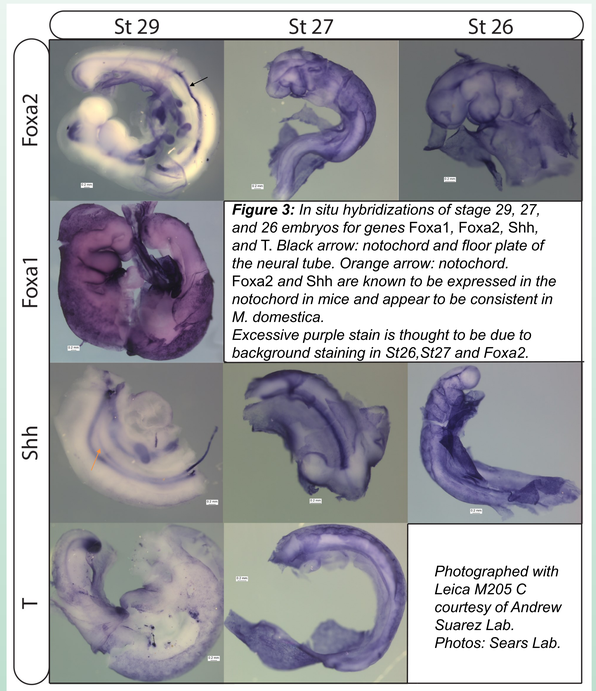


Figure 3: *In situ hybridizations of stage 29, 27, and 26 embryos for genes* Foxa1, Foxa2, Shh, *and* T. *Black arrow: notochord and floor plate of the neural tube. Orange arrow: notochord.*
Foxa2 *and* Shh *are known to be expressed in the notochord in mice and appear to be consistent in M. domestica.*
Excessive purple stain is thought to be due to background staining in St26,St27 and Foxa2.

Photographed with Leica M205 C courtesy of Andrew Suarez Lab.
Photos: Sears Lab.

Figure 3 continued: *Embryos were run through a four day protocol which bound a probe to mRNA of the target gene, attached an antibody to the probe, washed off excess antibody, and used an alkaline phosphatase enzyme on the antibody to develop BM Purple in locations that the gene was expressed.* Shh *plays a role in regulating disc formation.[6]* Foxa1 *and* Foxa2 *are transcription factors that regulate the formation of the notochord.[6]*

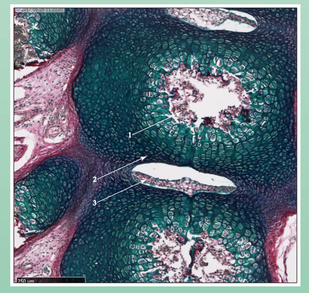


Figure 4: *Thoracic vertebra of a P10 opossum pup. Arrow 1: ossifying vertebra. Arrow 2: IVD. Arrow 3: nucleus pulposus of IVD.*

Vertebral column was dissected out, embedded in paraffin wax, sectioned in 7µm sections with a microtome and stained with Alcian Blue (mucopolysaccharides) and Picrosirius Red (collagen).[7] Photographed with Nanozoomer.

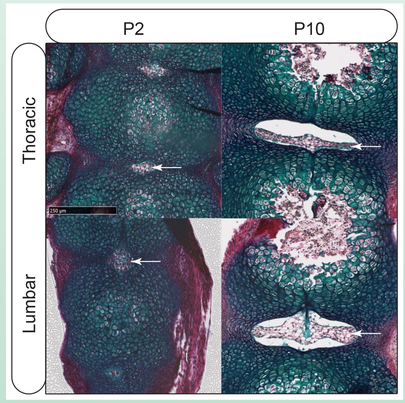
Project Description

In order to study the development of the notochord into the nucleus pulposus we embedded vertebral columns of pups 0-10 days old in paraffin wax and sectioned/stained them to observe the formation of the discs. We also did skeletal stains of pups 6-10 days old to visualize the maturation of the entire skeleton. We used whole-mount *in situ* hybridization (WISH) of embryos to characterize the genes expressed in the forming notochord. All animal work is in accordance with UIUC IACUC

Research

Preliminary data suggests that some genes involved in the formation and maintenance of the notochord in mice are the same in *M. domestica.* Preliminary data also suggests the ossification of the vertebral column of pups proceeds anteriorly to posteriorly and that much of the maturation of the nucleus pulposus happens in the first ten days.

Figure 5: *Thoracic and lumbar sections of opossum pups ages 2 days and 10 days after birth.*
White Arrows: nucleus pulposus.
The nucleus pulposus in the P2 vertebral columns is beginning to develop. By P10 the nucleus pulposus has developed considerably, suggesting much development occurs between these stages.
Preparation same as Figure 4.
Photographed with nanozoomer.



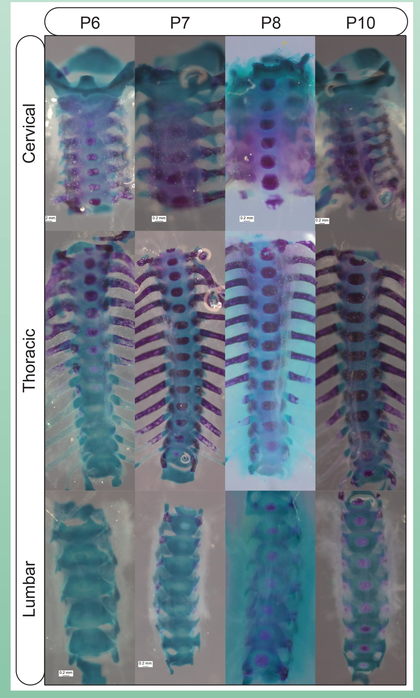


Figure 6: *Skeletal stains of cervical, thoracic, and lumbar sections of vertebral column from opossums pups 6,7,8, and 10 days old. Pups were skinned and stained with Alcian Blue (cartilage) and Alizarin Red (bone). Bone formation appears to advance anteriorly, from the mid thoracic region, posteriorly, to the lower lumbar region between the 6th and 10th day after birth.*
Photographed on Leica M205 C.

Conclusions

While much work is still needed, preliminary data appears to support our hypothesis that the vertebral column of *M. domestica* matures after birth in an anterior to posterior fashion.

Future Work

This work focused on the fate of the notochord. Once completed and expanded upon, it will help build the foundation for the potential use of *M. domestica* as a model animal for IVD research in the future. Once the timeline for the formation and major gene expression of the IVDs is characterized in *M. domestica*, researchers could potentially manipulate the formation of the IVDs. Future work would be needed to characterize the sclerotome of the somite, which is necessary for the formation of the annulus fibrosis and vertebrae.

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