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Research Article

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Gene Expressions and Development: The Pattern of VEGF Expression in the Developing Avian Spinal Cord

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Abstract

Introduction: *The development of the spinal cord is a complex process. It involves the coordinated action of multiple genes and specific environmental factors. While there are some differences between avian and mammalian species, the general processes of spinal cord development are conserved. Both species exhibit some similarities in gene expression patterns, which play a crucial role in patterning the spinal cord along the anterior-posterior and dorso-ventral axes. Developmental genes in neural specification, neurogenesis, and axon guidance, such as Sox, Pax, and Netrin, are also conserved between avian and mammalian species. The aim of this work is to ascertain the pattern of VEGF expression in the developing spinal cord of the domestic fowl, Gallus gallus variant domesticus.*

Methods: *With ethics clearance fertile eggs at stages 19-29 were incubated, routinely processed and sections of 5µm thickness were immunolocalized for VEGF A using immunofluorescence staining technique. Primary Antibody (Santa Cruz, Polyclonal rabbit anti-VEGF antibodies,1:100), Secondary Antibody (Santa Cruz, Anti-phosphotyrosine (4G10)–Alexa Fluor® 568 Goat Anti-Rabbit IgG; (Molecular Probes), 1: 300) were used. Nuclear stain was done with DAPI (Boehringer Mannheim), 1:10000. Positive control was performed using pregnant mouse uterus. Sections were viewed on an Olympus IX71 inverted fluorescent microscope and images captured using Olympus analysis software.*

Results: *VEGF was expressed in different cell types, including neural progenitor cells and endothelial cells.*

Discussion and Conclusion: *Vascular Endothelial Growth Factor plays a crucial role in the development of the spinal cord in vertebrates. Our work hereby presents a novel angle in the evaluation of VEGF expression in the spinal cord having been carried out at different stages of avian development. This is significant in that abnormal VEGF expression or function may contribute to developmental disorders of the spinal cord, such as neural tube defects (NTDs). Moreover, knowledge of VEGF expression pattern during spinal cord development may inform new therapeutic strategies for spinal cord injuries and diseases.*

Keywords: Spinal cord, VEGF, Gene expression, Perineural angiogenesis, Avian development

1. Introduction

The spinal cord is continuous with the brain, mediates spinal reflexes and is a site for sensory integration. It also provides the pathways between the brain and the rest of the body [1]. It is supplied by the anterior and posterior spinal arteries that descend in the pia from the intracranial part of the vertebral artery. These arteries are reinforced serially by branches from the ascending cervical, the cervical part of the vertebral, the intercostal and the lumbar arteries [2]. Development involves many distinct processes such as cell proliferation, cell migration, cell differentiation, neural innervation and vascularization. These events are regulated by growth factors that are present in the extracellular matrix. Spinal cord development involves coordinated action of multiple genetic and environmental factors. These genes or transcription proteins regulate early processes such as cleavage, gastrulation, morphogenetic cell movement and fate mapping (Brison et al., 2014). According to Nagai et al., clevage occurs in avian embryos before the eggs are laid with cell number increasing from 1 to about 2000, resulting in tissues of 5 to 6 cell thickness with tight junctions [3-5]. The blastodisc forms within 20 hours of fertilization [6]. While there are some differences between avian and mammalian species, the general processes of spinal cord development are conserved between these two groups [7, 8].

Conserved developmental Processes exist between avian and mammalian species.

Major developmental processes such as neurulation, pattern formation, synaptogenesis and gene expression are conserved in birds and mammals; both undergo neurulation, and their neural tubes eventually gives rise to the spinal cord [9]. Moreover, their spinal cords are patterned along an anterior-posterior and dorsal-ventral axis, with similar genetic mechanisms regulating the specification of neural progenitor cells [8]. Not to mention that these neural progenitor cells undergo neurogenesis and differentiate into various neuronal subtypes, including motor neurons and interneurons and their axons both grow and navigate to their targets, forming synapses and thus establishing functional neural circuits [10,11].

1.1 Pattern of VEGF Expression

VEGF is expressed in a spatial and temporal manner during spinal cord development, with highest expression levels during mammalian embryonic development [12]. This growth factor promotes angiogenesis that is essential for tissue perfusion; the delivery of oxygen and nutrients to the developing spinal cord for neurogenesis and neuronal survival. VEGF therefore has neurotrophic effects in the developing spinal cord [13, 14]. According to Ogunshola et al., VEGF may play a role in axon guidance and synaptogenesis [15]. Functional conservation of Despite species-specific variations and adaptations that reflect the unique characteristics of each organism, the general processes of spinal cord development such as neurulation, pattern formation, axon guidance and synaptogenesis are therefore conserved between mammalian and avian species [7, 8]. This work will contribute significant information to the management of developmental disorders of the spinal cord.

2. Methods

Animal ethics clearance was obtained (2008/7/1). Freshly laid, fertile eggs of the New Hampshire breed of chicken (*Gallus gallus variant domesticus*) of the same clutches, were obtained and incubated in thermostatically regulated incubators. The eggs were turned twice each day and humidity was maintained using sponges soaked in water baths that were placed in the incubators.

2.1 Tissue Processing Technique

Truncal segments of the embryos were fixed in 4% paraformaldehyde and then routinely processed, and paraffin sections of 5µm thickness were obtained for H and E staining. Every 10th section was then immunolocalized for VEGF A.

2.2 Immunofluorescent Staining Technique

Deparaffinized sections were permeabilized to facilitate antibody access. Immunofluorescence technique was used to detect and visualize VEGF proteins antigens in the tissues. Antibody concentration was predetermined through antibody optimization according to the manufacturer's protocol; Rabbit VEGF primary antibodies (VEGF, A-20: sc 152, Santa Cruz Biotechnology, Inc. USA, sc-2012, diluted with 0.1%BSA/PBS, 1:100 µl); the recommended dilution range was 1:100-1:400. The slides were then incubated in a moist chamber overnight in the fridge at 40 C. The sections were washed in three changes of 0.1% BSA/ PBS for 5 min each and 50µl of the secondary antibodies (Anti-phosphotyrosine (4G10) – (Alexa Fluor 568) diluted in PBS was added to the sections and incubated for 1 hour at room temperature after which they were washed in three changes of 0.1% BSA/PBS for 5 min each in foil covered glass containers and then rinsed in PBS. The nuclear stain (DAPI, dilution of 1: 100) was added to the sections for 10 min and rinsed off in PBS. The sections were mounted with cover slips using an aqueous mounting medium. Images were viewed on an Olympus IX71 inverted fluorescent microscope and captured using Olympus Analysis Software.

2.3 Control Sections

Negative sections were incubated with primary antibody but secondary antibody was replaced with 100µl of 0.1% BSA/PBS. Positive controls were performed on histological sections of pregnant mouse uterus (5µm thickness) [16, 17].

3. Result

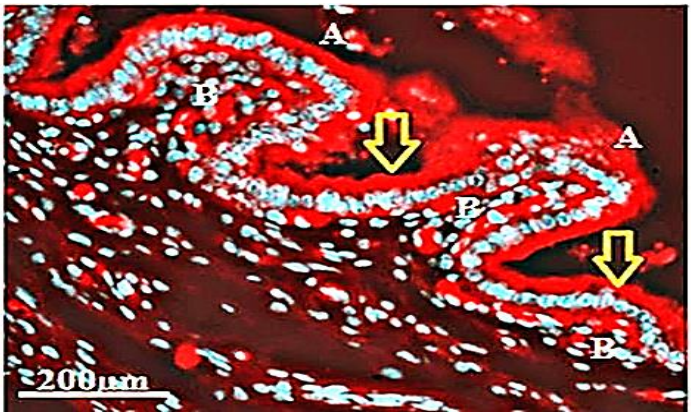

Figure 1: Positive control sections using pregnant mouse uterus. Endometrial epithelium (arrow heads) shows VEGF expression in both the apical (A) and basal (B) borders

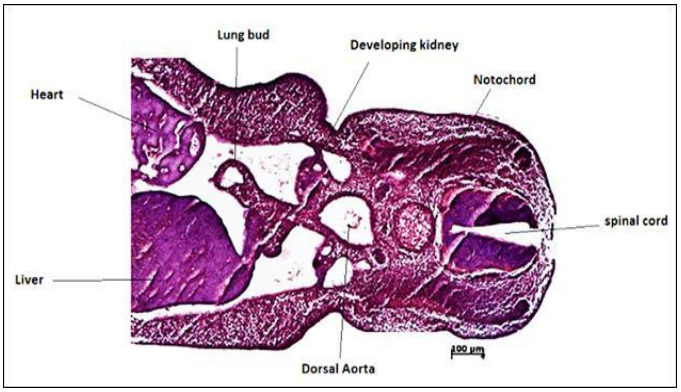

Figure 2: A cross section through a stage 22 embryo

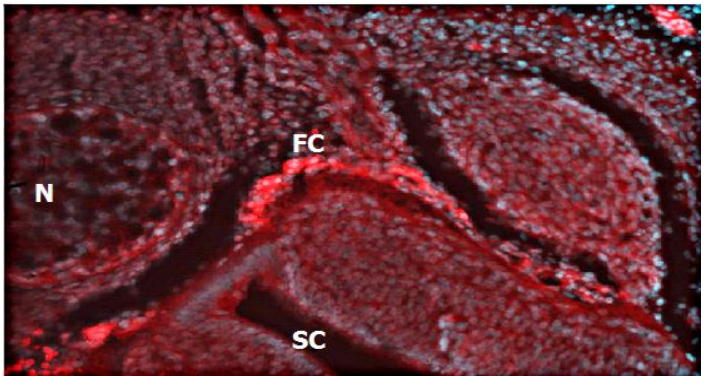

Figure 3: Figure shows the spinal cord at stage 25 of development (x400). Fluorescing cells (F) were seen around the spinal canal (SC) suggesting perineural angiogenesis. VEGF expression can also be seen in the endoneurium

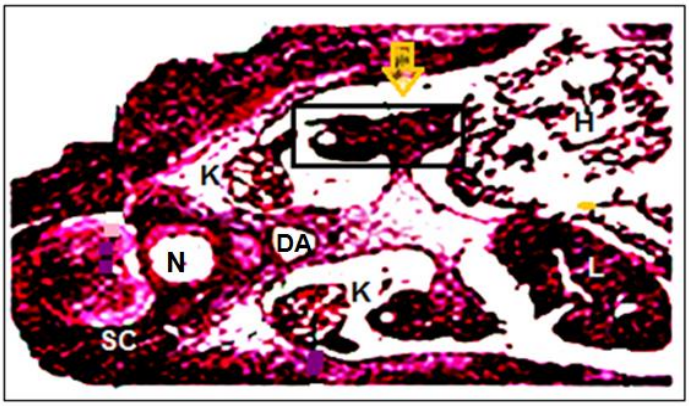

Figure 4: A figure showing structures in a stage 27 embryo; lung bud (highlighted), Heart (H), spinal cord (SC), liver (L), dorsal aorta (DA) and kidneys (K).

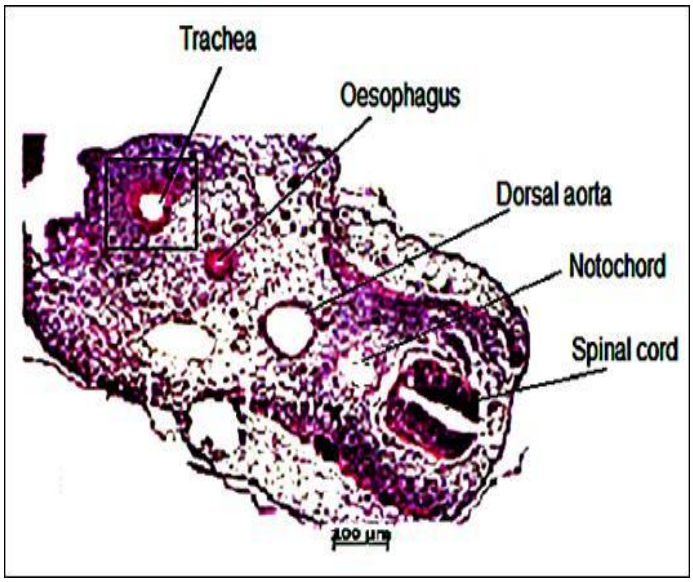

Figure 5: H and E section of the developing Spinal cord of stage 29 Embryo

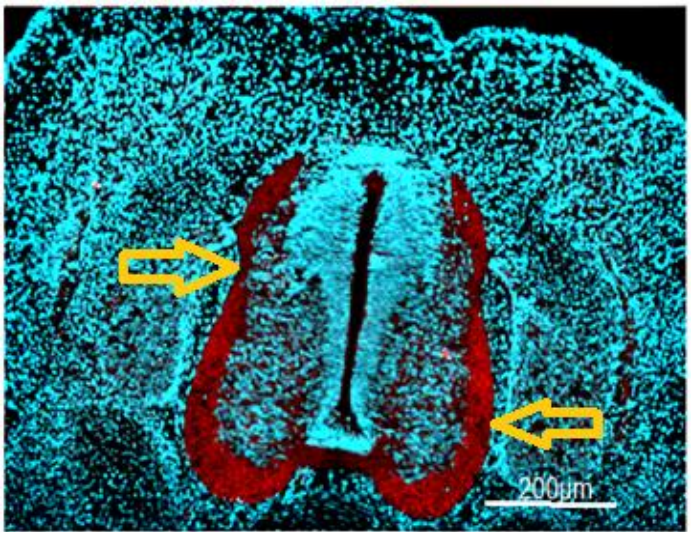

Figure 6: Spinal cord section of stage 29 embryo. There is a distinct VEGF expression in the perineural tissues (arrow head)

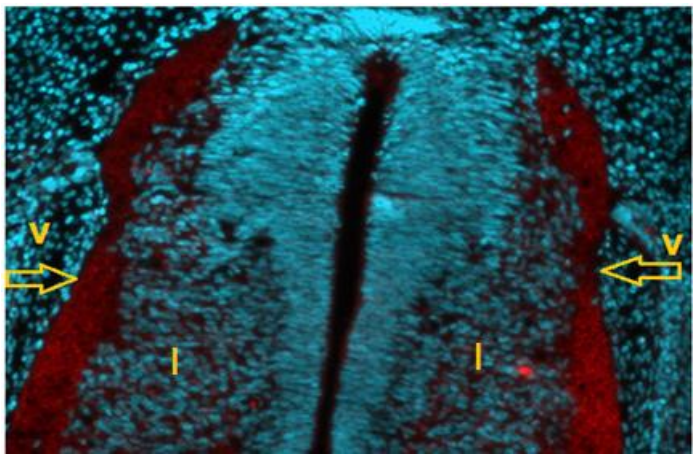

Figure 7: At a higher magnification of Stage 29 embryonic spinal cord (X400). There is diffuse angiogenic activity with vascular infiltration (I) of neural tissues (arrow head V).

4. Discussion

The development of the central nervous system of vertebrates starts with the formation of the neural tube, an ectoderm-derived embryonic structure that gives rise to the brain and the spinal cord [18]. Research from the last decade suggests that during development the nervous and vascular systems actively and vitally crosstalk with each other to build a fully functional organ system [19, 20]. The early development of the spinal cord vasculature begins around embryonic day 8.5 in mouse embryos and day 3 in avian embryos with the formation of the perineural vascular plexus (PNVP, a primitive vascular network formed via the process of vasculogenesis [21, 22]. Angiogenesis occurs as part of organogenesis and is a process where both blood vessels and blood cells are formed from the embryonic mesoderm. The Blood islands that are formed contain progenitor blood cells called

haematoblasts [23]. The assembling of any vascular bed initially requires proliferation, migration, and differentiation of mesoderm-derived angioblasts, a mesenchymal cell type giving rise to the endothelial cell lineage [21, 22]. The first key factors that contribute to this process (Fibroblast Growth Factor (FGF-2) and vascular endothelial growth factor VEGF), was initially identified by experimentation in quail [24, 25].

4.1 Pattern of VEGF Expression in Embryonic Tssues

There was no VEGF expression before stage 22 of avian development (3.5 days of incubation). VEGF is expressed in a spatial and temporal manner during spinal cord development, with the highest expression levels in the oldest embryo (day 14). This agrees with the work of Ferrara et al., [12]. We found cellular localization of VEGF was expressed in various cell types, including angiogenic cells, haematoblasts and neural progenitor cells in the perineural tissues. This aligns with the findings of Ogunshola et al., [15]. Furthermore, VEGF expression increased with embryonic age. According to Schoenwolf and Smith, [9], developmental timing differs between avian and mammalian species, in that avian embryos develop more rapidly. And while the overall organization of the spinal cord is similar, there are species-specific organizational and structural differences in certain regions of the spinal cord [26].

In this work we noted subcellular (cytoplasmic) localization of VEGF in diffuse pattern in the developing spinal cord. Krumlauf, found that both avian and mammalian species exhibited similar Hox gene expression patterns [27]. This suggests that these genes play a crucial role in patterning the spinal cord along the anterior-posterior axis. Furthermore, Sox, Pax, and Netrin genes regulated neural specification, neurogenesis, and axon guidance in the developing avian and mammalian spinal cords [8, 28]. From our work we can postulate that VEGF promotes angiogenesis, which is essential for the delivery of oxygen and nutrients to the developing spinal cord. This agrees with the work of Carmeliet [13]. VEGF has neurotrophic effects in avian neurogenesis and thus promotes neuronal survival in the developing spinal cord. This in agreement with the findings of Jin et al., (2000). This also implies that VEGF plays a role in axon guidance and synaptogenesis but the exact mechanism of action needs further exploration [15].

5. Conclusion

The functional role of VEGF in promoting angiogenesis and neurogenesis is conserved between avian and mammalian species, particularly, the general processes of spinal cord development. This reflects the shared evolutionary history of these two vertebrate groups. The conservation of spinal cord development between the two species makes avian models, such as the developing chick embryos of the domestic fowl (*Gallus gallus variant domesticus*), useful for studying spinal cord development and diseases [9].. This useful translational research proffers further understanding into the processes of spinal cord development and informs on possible development of therapeutic strategies for spinal cord injuries and diseases [29-31].

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Recommendation

This work can be done on a larger scale and in different vertebrate species.

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