Genetic Regulation and Renal Development: Vascular Endothelial Growth Factor A (VEGF-A) Expression in Early Avian Kidney Systems

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Abstract-

Introduction: ***Like in mammals, the renal kidney develops through three successive slightly overlapping systems; pronephros, mesonephros and metanephros. The pronephros develops from the surrounding mesoderm but gets replaced by the mesonephros around days 3–4 of incubation, functioning from days 5 to 11 before degenerating at around embryonic day 15 as it gets replaced by the definitive metanephric kidney.***

Renal development across vertebrates involves a complex interplay of genetic and molecular mechanisms; a complex series of morphogenetic events, lead to formation of renal tubules, glomeruli, and collecting ducts. In order to ensure both water and electrolyte homeostasis in the avian embryo that is contained within its egg, the developing metanephric kidneys works in harmony with the chorioallantoic membrane (CAM). Angiogenesis is a major component of kidney development. Vascular endothelial growth factor (VEGF) initiates renal vascular development, thus acting as a regulator of kidney development and function (Ferrara et al., 2003).

Aim: This study investigates VEGFA expression in avian kidney progenitor cells and its potential role in the first week of avian kidney development .

Methods: Fertile eggs of the domestic fowl were incubated at 38 °C with relative humidity of 55–60% and were examined days at day 3–7 of development. Using routine immunofluorescent technique, parrafin sections of embryonic truncal regions at the different developmental stages were examined for VEGFA expression in avian kidney progenitor cells.

Results: VEGF was expressed by early kidney progenitor cells (pronephros) from day 3 of development. By day 5-6 of incubation, renal tubular cells showed marked apical expression of VEGF.

Discussion and Conclusion: Angiogenesis is vital to the development of the kidney's complex vascular network, that is essential for the organ's function of blood filtration and urine production. Our results show that VEGFA is expressed in avian kidney progenitor cells from day three of incubation. These cells are localized in both the developing renal vasculature and tubules thus promoting angiogenesis and tubulogenesis. This early tubular development is significant considering their role in reabsorption and secretion of substances.

VEGFA signaling pathways involve binding to VEGFR, thus activating downstream signaling pathways, and regulating endothelial cell survival, proliferation, and migration. Furthermore, exogenous recombinant human VEGF (rhVEGF) to kidney explants induced differentiation and proliferation of endothelial cells, resulting in vasculogenesis and tubulogenesis. We found the high VEGF expression levels occurred during early stages of avian development; 22-27. This coincides with both the pronephric and mesonephric stages of renal development. Interference to these functions may be the underlying cause in the pathophysiology of some renal diseases.

Index Terms- vertebrates, gene expression, VEGF, Renal angiogenesis, tubulogenesis

I. INTRODUCTION

Vertebrate renal development is rather complex in that the processes involve coordinated action of multiple genetic and molecular mechanisms, some of which are conserved across vertebrate species e.g. genetic regulation [1], molecular signaling pathways [2] and morphological similarities [3]. Kidney development in vertebrates involves a complex series of morphogenetic events, including the formation of renal tubules, glomeruli, and collecting ducts. While the processes differ across species, it generally follows a similar pattern [3].The pronephros is the first stage of kidney development that comprises a simple, temporary excretory and osmoregulatory structure[4]. The second stage kidney or mesonephros, is the primary excretory organ in fish and amphibians unlike in more advanced vertebrates (such as amniotes) in which during embryogenesis, it is the second stage kidney that becomes replaced by the metanephros or definitive kidney [3]. The metanephros develops from the interaction between the ureteric bud and metanephric mesenchyme and forms the permanent kidney in reptiles, birds, and mammalst [5].

II. ANGIOGENESIS AND VASCULAR MORPHOGENESIS

Angiogenesis involves the formation of vascular sprouts from pre-existing vessels with resultant formation of a highly branched vascular plexus. It is a fundamental process in both development [6] and wound healing. Under these conditions, the process of angiogenesis is highly regulated [7] being a complex multi-step process involving the release of proteases from "activated" endothelial cells, breakdown of the basement membrane and migration of the cells to the interstitial space, where they undergo proliferation. The cells then acquire a lumen and pericytes are recruited for vascular remodeling, after which a new basement membrane is formed. The newly formed vessels then fuse leading to initiation of blood flow [8,7]. Furthermore, angiogenesis plays a crucial role in maintaining tissue blood supply and oxygenation and so, a poor angiogenic process can lead to various pathological conditions. Anti-angiogenic factors play a crucial role in regulating angiogenesis; in cancer, angiogenesis is often dysregulated, promoting tumor growth and metastasis [9]. Angiogenesis inhibitors, such as VEGF receptor (VEGFR) inhibitors, have therefore been developed to target such pathological processes.

III. STRUCUTRE AND MOLECULAR REGULATION OF KIDNEY DEVELOPMENT

Key Differences Across Vertebrate kidney lies in numerical variation of nephrons, glomeruli, and collecting ducts [10] developmental timing [4] and functional adaptation to their respective environmental conditions such as freshwater or saltwater that necessitate development of unique features needed to maintain osmoregulation [10]. Genetic factors such as Wilms tumor-1 (WT1), glial cell line-derived neurotrophic factor-RET (GDNF-RET) regulate renal development and differentiation [5] .and the GDNF-RET complex signaling pathways have been associated with ureteric bud growth and branching morphogenesis [2]. VEGFA has been immunolocalized in the developing kidney [11] and administration of angiogenic growth factors, such as VEGF, promotes angiogenesis and improve tissue blood supply [12]. Not to mention that cell-based therapies, like stem cell transplantation promote angiogenesis and thus improve tissue blood supply and tissue perfusion [13]. Gene therapy can be used to deliver angiogenic genes to specific tissues, thus promoting angiogenesis and improving tissue blood supply [14].

IV. THERAPEUTC USES OF ANTI-ANGIOGENIC FACTORS

Endogenous anti-angiogenic factors such as thrombospondin-1 (TSP-1), have been shown to inhibit angiogenesis by binding to endothelial cells and inducing apoptosis [15]. And exogenous anti-angiogenic agents, such as bevacizumab, a monoclonal antibody targeting VEGF, have demonstrated efficacy in treating colorectal and breast cancer [12]. The therapeutic potential of anti-angiogenic factors lies in their ability to starve tumors of oxygen and nutrients, and so slowing

growth and metastasis. However, resistance to anti-angiogenic therapy can develop, highlighting the need for further research into combination regimens and novel targets [16]. Nevertheless, anti-angiogenic factors offer a promising approach to cancer therapy, and ongoing research aims to optimize their use and overcome resistance.

V. METHODS

Care of The Embryo

Animal ethics clearance was obtained (Ethics clearance certificate number 2008/7/1) and 42 freshly laid, fertile eggs of the New Hampshire breed of chicken (Gallus gallus variant domesticus) and of the same clutches, were incubated in a humidified thermostatically regulated incubator set at 38^0C. The eggs were turned twice each day and treated on day 3 of incubation (expected Hamburger and Hamilton's stage 21

Tissue processing technique

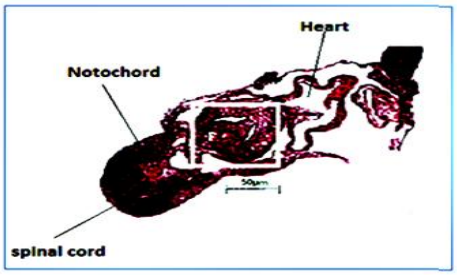
The egg shells were cracked, and the embryo harvested and immersed in chick ringer inside an egg bowl. The embryos were then placed in a wax dish, staged using Hamburger and Hamilton's staging for avian embryos. The trunk was resected out and fixed in 4% paraformaldehyde overnight and then processed in an automatic tissue processor (Shandon citadel 1000).

VEGF Immunolocalization

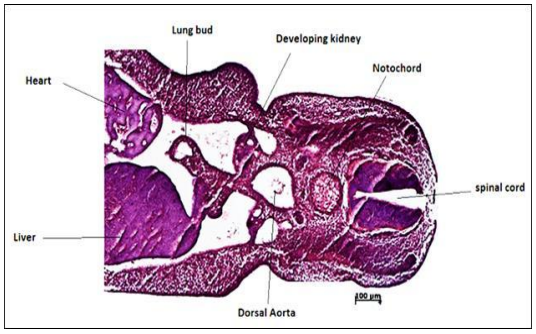
Paraffin sections of 5 µm thickness were immunolocalized for VEGF using standard immunofluorescence: sections were deparaffinization and rehydrate through a series of ethanol washes and microwave technique was used for antigen retrieval. Bovine serum albumin (BSA) was used for blocking non-specific binding sites after which the tissues were incubated with primary antibody overnight (polyclonal goat anti-rabbit antibodies 1:100 dilutions and detected with fluorescently labeled secondary antibody (secondary Alexa Fluor® 568 red goat anti-rabbit IgG at 1:300 dilution, Santa Cruz). Nuclear staining was done with 1:1000 dilutions of DAPI. The sections were mounted with antifade medium and coverslip, and visualized using an Olympus IX71 inverted fluorescent microscope. Images were captured using Olympus analysis Software. Pregnant mouse uterine tissues were used as positive controls and negative controls were performed on sections adjacent to the test sections, but using phosphate buffered saline (PBS) in place of the secondary antibody.

VI. RESULTS

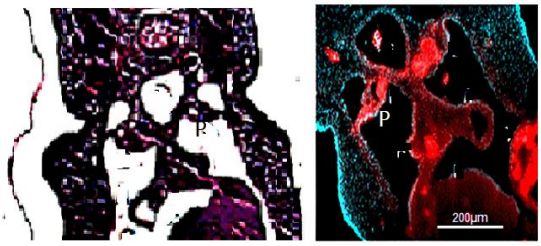
From as early as stage 22 (Day 3.5) of development, VEGF immune-reactivity was found in multiple organs of the developing embryos; kidneys, heart, lung, the spinal cord and embryonic vessels.



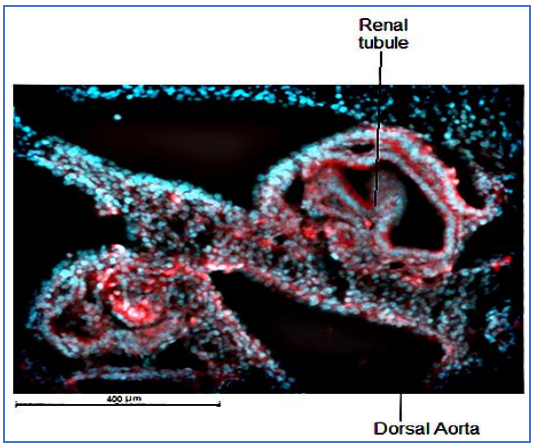
Stage 20 Embryo: No sign of renal development at this stage



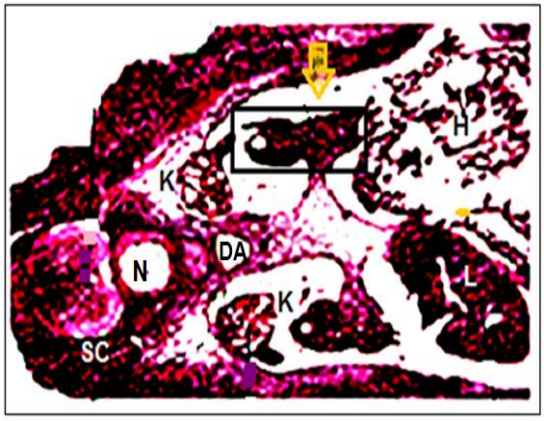
Stage 22 Embryo. A cross section through a stage 22 embryo. The right lung buds are distinctly formed and lie just ventral to the developing kidney.



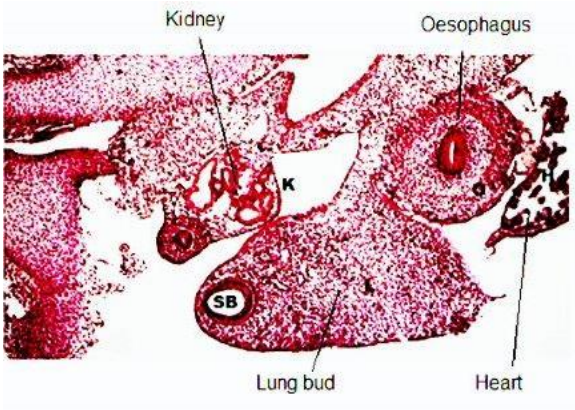
Stage 22 embryo: Developing pronephros. Note early VEGF expression



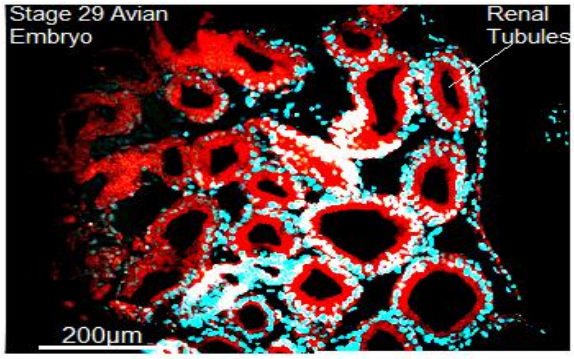
At Stage 22 (Day 3 of incubation), the developing pronephric tubules show marked VEGF eexpression in renal progenitor cells.



Stage 27 embryo: Showing lung buds (highlighted), Heart (H), spinal cord (SC), liver (L), dorsal aorta (DA) and the mesonephric kidneys (K).



Stage 29 (Day 6 of incubation) showing the developing mesonephric kidneys (K). Note the lung buds lying just ventral to the oesophagus and a section through the secondary bronchi (SB)



Stage 29 embryo (Day 6 of incubation): Note the developing mesonephic tubules show marked apical tubular VEGF expression

VII. DISCUSSION

The kidney, heart and brain are perticularly sensitive to reduced cardiac output, changes in which can significantly impact bodily functions. The kidneys play a crucial role in regulating blood pressure and fluid balance [17]. While there are key differences in kidney development across species, some features are conserved; genetic regulation, molecular signaling pathways, and morphological similarities. Understanding these conserved features can provide insights into the mechanisms of both kidney development and diseases. And while the stages of kidney development are similar in vertebrates, differences in kidney structure, developmental timing, and functional adaptation reflect the renal diversity of vertebrate species. We found that VEGF was expressed by angioblasts, blood cells and in renal tubular cells that usually are involved in reabsorption and secretion of substances in the kidney. This agrees with the findings in the mammalian kidney by[18]. During avian development, the metanephric or definitive kidneys and chorioallantoic membrane (CAM) work in harmony, thus ensuring water and electrolyte homeostasis within the egg [19]. There are different types of endothelia in the kidney and each has specific structural and functional characteristics. Although both the glomerular and peritubular endothelium are fenestrated, glomerular epithelium does filtration and peritubular endothelium carries out transportation and reabsroption of substance in the glomerular filtrate while also performing normal physiological epithelial function [20]. This function is an important factor in the evaluation of the

progression of renal diseases; it also serves as a prognostic parameter for ascertaining occurrence of seconday renal pathologies e.g secondary to cardiovascular disorders [21].

VIII. PRO-ANGIOGENIC, ANGIOGENIC, ANTI-ANGIOGENIC FACTORS AND DISEASE

An imbalance between pro-angiogenic and anti-angiogenic factors can disrupt the angiogenic process [22] while dysfunction of these cells impair the angiogenic process [23] e.g. chronic inflammation inhibits angiogenesis by promoting release of anti-angiogenic factors [24]; inadequate blood supply leads to tissue hypoxia, resulting in cellular damage and dysfunction [25]. Furthermore, poor angiogenesis can impair wound healing by reducing blood supply to the affected area [26]. This phenomenon has contributed to the occurrence of atherosclerosis and heart failure [22]. Understanding the causes and consequences of poor angiogenesis can help in the development of therapeutic strategies to promote angiogenesis and improve tissue blood supply and tissue perfusion. VEGF has been implicated in many kinds of kidney diseases, presenting itself as a foe. But studying its role has thrown more light into understanding pathologies such as polycystic kidney disease, cancers and diabetic retinopathy, which on the other hand presents VEGF as a friend. From our work we can conclude that VEGFA regulates early kidney development and function and its expression in avian kidney progenitor cells seems to be essential for developing kidney vasculature, tubulogenesis thus, regulating renal function. This study presents VEGF expression in the kidney as a potential target for disease control, and have important implications for understanding the pathogenesis of renal diseases.

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