DEVELOPING AND MATURE NEPHRONS OF ADULT DOGFISH *Squalus acanthias* EXPRESS VEGF-LIKE PROTEIN

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Vascular endothelial growth factor (VEGF) is a central regulator of angiogenesis and vasculogenesis in mammals (for review see Neufeld, G. et al. FASEB J. 13:9-22, 1999, Ferrara, N., J. Mol. Med. 77:527-543, 1999). During organogenesis of the kidney, the development of the nephron is tightly coupled to the formation of blood vessels. The differentiation of fenestrated endothelia of the glomerular capillary convolute and the peritubular circulation is controlled by the cooperation of VEGF and its receptors. A specific receptor is Flk-1, which is a marker for angioblasts. In the development of the murine metanephros, angioblasts are able to build the entire microcirculation by vasculogenesis (Robert B. et al., Am.J.Physiol. 275:F164-F172, 1998). In the mature nephron of mammals, VEGF is present in podocytes of the glomerulus and the epithelial cells of collecting ducts.

Elasmobranch fish provide animal models for the simultaneous study of developing and mature nephrons, which lie side by side in their kidneys (Hentschel H., Am.J.Anat. 190:309-333, 1991, Elger M. et al., this bulletin). The aim of the present study was the histochemical detection of VEGF- and Flk-1-like protein in the kidney of spiny dogfish, *Squalus acanthias*. For the localization of developmental stages in zones of nephroneogenesis, we prepared serial cryosections of perfusion-fixed kidneys of adult female spiny dogfish. Sections were incubated with anti-VEGF- and anti-Flk-1-antibody (Santa Cruz). Binding sites were revealed with ABC-elite kit (Vector Laboratories, Burlingame).

Fig. 3 shows a western blot from shark heart, kidney, rectal gland, liver and testis, hybridized with a human anit-VEGF antibody. In each lane, we observed several bands. The antibody is directed against the human VEGF 165 corresponding to the 39 kDa band. The other bands may be due to different VEGF isoforms or VEGF dimers rather than to glycosylation products. Using the recombinant VEGF protein as antibody inhibitor, all bands became weaker except the 32 kDa band from testis (data not shown). Our western blot data demonstrate that the VEGF antibody reacts specifically with VEGF isoforms expressed in kidney. Therefore, this antibody is well suited for following immunhistological stainings.

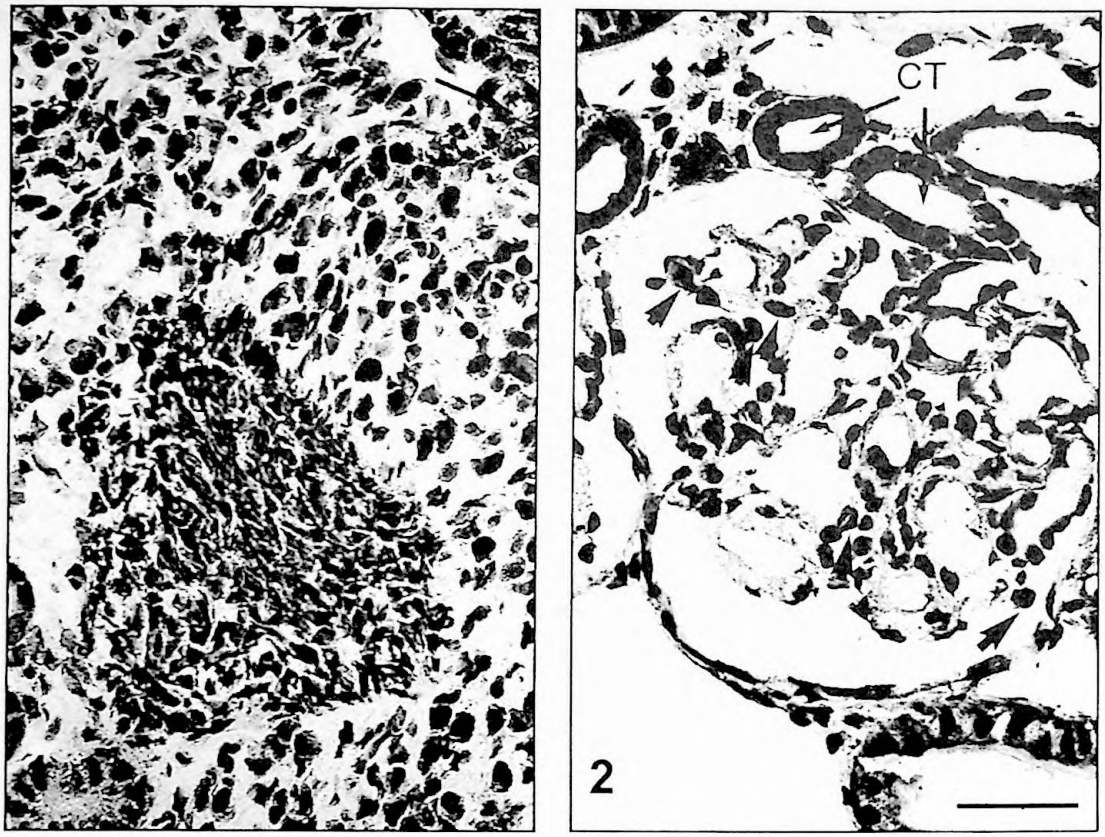

Fig. 1. Cross section through a nephron developmental stage I (condensed mesenchym). The outer cell layer of the mesenchymal mass is labelled by VEGF-antibody. Counterstain is hematoxylin. Calibration bar (see fig. 2) equals 25 µm. Fig. 2. Cross section through a glomerulus. Collecting tubule (CT) cells at the vascular pole distinctly react with antibody. The cytoplasm of many podocytes is labelled (arrows). Calibration bar for this figure equals 15 µm.

All stages of nephron development were found in the cell-rich interstitial tissue in the vicinity of blind ends of the collecting tubule-collecting duct system. Four stages of nephron development were observed. At stage I the nephron was characterized by a condensed mass of mesenchymal cells in the center of concentrical layers of connective tissue. At stage II an elongate cyst with an s-shaped bend and a high-prismatic epithelium was present. This s-shaped body was connected by a developing collecting tubule with the collecting duct system. At stage III, the developing nephron possessed the essential features of the mature nephron (glomerulus, tubular segments segregated into mesial tissue and lateral bundles), but complete differentiation was lacking. Developmental stage IV, the young nephron, was similar to the mature nephron, however, glomeruli and tubular segments were smaller than those of mature nephrons.

VEGF-like immunoreactivity was observed in epithelial cells of all collecting ducts, including the blind ends of developing collecting tubules, collecting tubules of developmental

stages II to IV, and collecting tubules of mature nephrons. Several podocytes of young and mature glomeruli reacted also (fig. 1). The outermost mesenchymal cells of the condensed mass (stage I) displayed distinct labeling (fig. 2). VEGF-like binding was seen in a large number of cells in the cell-rich connective tissue around the stages I. The majority of these cells resembled blast cells. Faint to moderate binding of anti-Flk-1 was also observed in blast-cell-like cells. These cells formed streaks which were frequently oriented perpendicularly towards the mesenchymal mass of stage I.

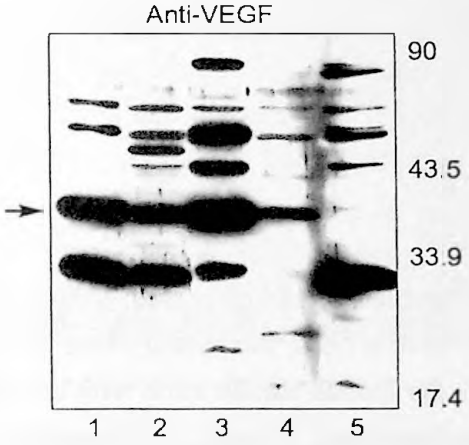

Fig. 3. Western-blot analysis of differnt shark tissues. 30ug protein from heart (lane 1), kidney (lane 2), rectal gland (lane 3), liver (lane 4) and testis (lane 5) were loaded. An antibody directed against the human VEGF 165 isoform was used. This antibody recognizes not only the 39 kDa VEGF-165 band, but also several other unknown VEGF isoforms from shark.

It is tempting to speculate that VEGF is produced by collecting duct cells in the vicinity of stage I and by the outer cell layer of the mesenchymal mass. A gradient of diffusible protein would build up in the extracellular matrix around the mesenchymal mass, directing motile cells (blast cells, mesenchymal cells) towards stage I and initiating the development of capillaries by the formation of endothelial tubes. In several mammalian systems VEGF-production is stimulated by hypoxia. This apparently is most likely also the case in *Squalus*, because the nephrogenic stages I are situated in a defined zone without capillary supply in the cell-rich interstitium. The presence of VEGF-like immunoreactivity in podocytes and collecting tubule cells of mature nephrons probably is needed for the maintenance of fenestrated endothelia, like in mammals. With financial support by DFG.