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Involvement of FGF and BMP family proteins and VEGF in early human kidney development

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Summary. The spatial and temporal pattern of the appearance of the fibroblast growth factor proteins (FGF-8 and FGF-10), the bone morphogenetic proteins (BMP-2/4 subfamily and BMP-7) and the vascular endothelial growth factor protein (VEGF) was investigated in the human mesonephros and metanephros of the 5-9 week-old conceptuses. In the mesonephros, both FGF's and BMP's were found in all structures and their expression slightly decreased in the early fetal period. VEGF positivity appeared in all mesonephric structures, and increased in the fetal period coincidently with formation of the mesonephric blood vessel network. In the metanephros, FGF-8 first appeared only in the metanephric mesenchyme, but from the 7th week on, its reactivity increased and spread to other metanephric structures. FGF-10 positive cells appeared in all metanephric structures already in the 5th week, and slightly intensified with progression of development. Cell survival and nephrogenesis in the permanent kidney might be associated with the appearance of both growth factors. Both BMP-2/4 and BMP-7 displayed a similar pattern of reactivity in all metanephric structures, and their reactivity intensified with advancing development. Alterations in their pattern of appearance might lead to the formation of small and dysplastic kidneys. Already in the earliest developmental stages, VEGF protein appeared in all metanephric structures. At later stages, VEGF showed more intense reaction in the collecting system than in the differentiating nephrons and interstitium. Due to VEGF involvement in vasculogenesis and angiogenesis, abnormal VEGF appearance might lead to impaired formation of the blood vessel network in the human permanent kidney.

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Introduction

The mesonephros is a transitional, but functional kidney that develops late in the 4th developmental week and is formed of a large number of s-shaped loops. The lateral ends of these loops enter the mesonephric (Wolffian) duct, while the medial end forms the Bowman's capsule surrounding the glomerulus. The mesonephros undergoes gradual degeneration from 8th to 16th developmental week.

The metanephros begins to develop in the fifth week of development when the ureteric bud penetrates the metanephric mesoderm. Under the inductive influence of the terminal branches (ampullae) of the ureteric bud, metanephric mesoderm condenses and covers the ampullae to form metanephric cups. During further development, metanephric cups give rise to the renal vesicles, s-shaped tubules (nephrons) and renal corpuscles (consisting of Bowman's capsule and glomerulus). The metanephros is a permanent kidney, which later in development transforms into more mature nephrons and a collecting system consisting of the collecting tubules, renal calyces, renal pelvis and ureter (Saxen, 1987; Sadler, 2004).

Fibroblast growth factors (FGF) are involved in cell proliferation, migration and differentiation of different structures and organs during the mammalian development (Ornitz et al., 1996; Celli et al., 1998; Ohuchi et al., 2000). Previous studies on FGF-8 mostly emphasized its involvement in chick and mouse limb development (Heikinheimo et al., 1994; Mahmood et al., 1995; Vogel et al., 1996). However, the expression of FGF-8 was found in the metanephric glomeruli of the mouse embryo (Mahmood et al., 1995), while FGF-8 mRNA was detected in the chick mesonephros as well

(Vogel et al., 1996). In the recent study performed by Grieshammer et al. (2005) on developing mice kidney, severely reduced FGF-8 signaling resulted in abnormal nephron formation, while in the complete absence of FGF-8, the nephron progenitor cells underwent apoptosis and no s-shaped nephrons were formed.

In the isolated rat ureteric bud culture, FGF-10 (and FGF-1) stimulates ureteric bud cells to form long, branching tubular structures with clearly formed ampullae (Qiao et al., 2001). Ohuci et al. (2000) reported smaller kidneys with dysplastic outer medulla in the FGF-10 null mice, indicating the importance of FGF-10 in the kidney growth.

Bone morphogenetic proteins (BMP) are the largest subfamily of the transforming growth factor ß (TGF-ß) superfamily of secreted proteins (Martinez et al., 2002). There are three BMP subgroups, with approximately 90% amino acid identity within the subgroup (Hogan, 1996; Raatikainen-Ahokas et al., 2000). BMP-2, BMP-4 and BMP-7 show dynamic expression patterns during the development of mouse kidney and urinary tract (Dudley and Robertson, 1997; Piscione et al., 1997, 2001; Godin et al., 1999; Raatikainen-Ahokas et al., 2000; Myazaki et al., 2003; Nakano et al., 2003). BMP-2 and BMP-4 form one of the BMP subgroups (Hogan, 1996) and have different expression patterns in the developing mouse metanephros (Dudley and Robertson, 1997). Piscione et al. (1997, 2001) suggested that in mice, BMP-2 controls ureteric branch formation by inhibiting the formation of cellular processes. Miyazaki et al. (2000, 2003) proposed that BMP-4 prevents cell death and inhibits cell condensation in the mice metanephric mesenchyme, regulates the site of initial ureteral budding on the Wolffian duct and promotes the growth and the elongation of the ureter. Both BMP-2 and BMP-4 homozygous null mutant mice died prior to metanephric development, whereas only BMP-4 heterozygous mice had renal abnormalities such as cortical cysts, hydronephrosis and other urinary tract abnormalities (Zhang and Bradley, 1996; Dunn et al., 1997; Miyazaki et al., 2000; Martinez et al., 2002). In the study on human embryonic aorta-gonad-mesonephric region, BMP-4 was found in the mesonephros and was suggested to have a role in human hematopoesis (Marshall et al., 2000).

BMP-7, a member of another BMP subgroup, was detected in developing mouse metanephros (Dudley et al., 1995; Luo et al., 1995; Vukicevic et al., 1996; Dudley and Robertson, 1997). BMP-7 signaling might be required for survival and for maintaining of the undifferentiated cell population in the metanephric mesenchyme by suppressing apoptosis and inhibiting tubulogenesis, thus enabling further kidney growth (Dudley et al., 1995, 1999; Piscione et al., 1997, 2001; Godin et al., 1999). BMP-7 defficient mice had small dysgenic kidneys with hydroureters (Luo et al., 1995; Jena et al., 1997; Dudley and Robertson, 1997). During human metanephric kidney development, BMP-7 mRNA was first detected at 6 weeks of gestation in the metanephric mesenchyme surrounding the ureteric bud. At later developmental stages, the highest levels of BMP-7 mRNA were detected in the developing glomeruli, whereas BMP-7 protein expression was found mostly in convoluted tubules (Vukicevic et al., 1994; Helder et al., 1995).

Vascular endothelial growth factor (VEGF) is essential for vasculogenesis and for the sprouting of new capillaries from preexisting vessels (angiogenesis) (Kim and Goligorsky, 2003; Makanya et al., 2005). VEGF was detected in mice and rat metanephros, where it was suggested to be involved in providing capillary formation and spatial direction toward forming nephrons, as well as in proliferation of tubular epithelia (Loughna et al., 1997; Tufro et al., 1999; Tufro, 2000). Disrupted vasculogenesis, angiogenesis and vascular spatial organization were found in VEGF deficient mice (Carmeliet et al., 1996; Ferrara et al., 1996; Ferrara, 1999). In human fetal kidneys, VEGF receptors (KDR and Flt-1) mRNA and protein were detected in the glomeruli and in capillaries and veins. VEGF mRNA and protein found in both mesonephric and metanephric glomeruli, were detected also in the collecting ducts, while only VEGF mRNA was present in the S-shape nephrons (Simon et al., 1995, 1998; Kaipainen et al., 1993).

The studies on the FGF-8, FGF-10, BMP-2 and BMP-4 involvement in kidney development were performed only on experimental animals, while the investigations on the role of VEGF and BMP-7 in the human developing kidney were done mostly on the human fetal tissue. In this study we investigated the spatial and temporal distribution pattern of FGF-8, FGF-10, BMP-2/4 subgroup, BMP-7 and VEGF in human embryonic and early fetal mesonephros and metanephros. We speculate on the possible role of these growth factors in the formation of nephrons, collecting system and renal vasculature in early human kidney development.

Materials and methods

Human material

A total of 8 normal human conceptuses between the 5th and the 9th developmental week were collected after spontaneous abortions from the Department of Gynecology and Obstetrics, Clinical Hospital Split, Croatia, and after the tubal pregnancies from the Department of Pathology, Clinical Hospital Split. The embryos and fetuses were examined macroscopically and measured. Only normal conceptuses, without any sign of abnormality, signs of intrauterine death or macerations were used in our study. The embryonic tissues were treated as postmortem material with permission of the Ethical and Drug Committee of the Clinical Hospital Split, in accordance with the 1964 Helsinki Declaration. The postovulatory age was estimated on the basis of the menstrual data and

correlated with the crown-rump length (CRL) and Carnegie stages (O'Rahilly and Gardner, 1971) (Table 1).

Immunohistochemical staining

Caudal parts of embryos containing developing kidneys were dissected. Tissue samples were fixed in 4% paraformaldehyde in phosphate buffer and dehydrated in 100% ethanol. They were embedded in paraffin wax, serially sectioned at 4-6 µm, mounted on glass slides, and analyzed using an Olympus BX-40 light microscope (Olympus, Tokyo, Japan). The shape of each developing structure in the mesonephric or metanephric tissue was analyzed using successive serial sections. This method allowed us to distinguish between part of the collecting system and different stages of nephron formation.

Sections were deparaffinized in xylene and rehydrated in ethanol and water. In order to quench endogenous peroxidase activity, sections were incubated for 10 minutes in 1% H_2O_2.

Sections for FGF-8, FGF-10 and BMP-2/4 antigen staining were incubated with anti-goat serum (X0907, DakoCytomation, Glostrup, Denmark) for 20 minutes in the dark. Sections were then washed in PBS and incubated with goat anti-FGF-8b primary antibody (AF-423-NA, R&D Systems, Minneapolis, MI, USA; concentration 15 µg/mL), anti-human FGF-10 antibody (AF345, R&D Systems, Minneapolis, MI, USA; concentration 15 µg/mL) and goat anti-BMP-2/4 antibody (AF355, R&D Systems, Minneapolis, MI, USA; concentration 10 µg/mL) for 45 minutes in the dark.

For BMP-7 staining, after washing in PBS, sections were immediately incubated with mouse monoclonal anti-human BMP-7 antibody (MAB3541, R&D Systems, Minneapolis, MI, USA; dilution 1:10) for 45 minutes in the dark.

After washing in PBS, sections for VEGF staining were incubated in EDTA for 10 minutes at 95°C. After cooling to room temperature, sections were incubated with rabbit anti-VEGF primary antibody (PC37, Calbiochem, USA; dilution 1:20), for 45 minutes in the dark.

Binding of FGF-8, FGF-10, BMP-2/4 and BMP-7 primary antibodies was detected using streptavidin-biotin peroxidase system (K0690, DakoCytomation, Carpinteria, CA, USA) as recommended by the manufacturer. Rabbit ABC Staining System (sc-2018, Santa Cruz Biotechnology, Inc., Santa Cruz, California, USA) was used for detection of VEGF primary antibody binding.

Afterwards, all sections were washed with PBS and then stained with diaminobenzidine solution (DAB). Finally, sections were rinsed in destillated water, counter-stained with hematoxylin, and dehydrated in ethanol and xylol. Analysis was performed on an Olympus BX-51 microscope equipped with DMP digital camera and using DP-SOFT Version 3.1 software. Three observers with consideration of inter-observer variation analyzed the labeling.

Controls

Sections without primary antibodies incubation were used as negative controls. Positive controls were developing kidney structures or other tissues in the same sections (as each section contained various types of tissues and organs) that were known to label specifically with primary antibodies. All antibodies used were obtained from respected commercial brands and when antibody data sheets were checked, no specific cross-reactivity was ever reported.

Semi-quantification

The intensity of labeling of the kidney tissue was semi-quantitatively selected into 4 categories according to the staining reactivity: absence of any reactivity at high magnifications (x100) = - ; mild reactivity - clearly seen at higher magnifications (x40) = + ; moderate reactivity - seen already at lower magnifications (x20, x 10) = + + ; and strong reactivity seen as a clear signal at the lowest magnification (x4) = + + +.

Results

Between the 5th and 6th week of development, the mesonephros consists of renal glomeruli and tubules opening into the mesonephritic duct (Wolffian duct) at the lateral side and forming the Bowman's capsule at its medial extremity. In the metanephros, the anterior actively growing portion of the ureteric bud (ampulla) induces surrounding cells of the metanephric mesoderm to proliferate and condensate to form the metanephric cup. The remaining cells of the metanephric mesoderm form the loose metanephric mesenchyme.

During the described developmental period, all mesonephric structures contain both FGF-8 and FGF-10 positive cells, with slight differences in staining intensity (Table 2, Fig. 1A). In the metanephros, FGF-8 positivity is found only in the metanephric mesenchyme, while FGF-10 positivity is present in all metanephric structures, especially in the ureteric bud and undifferentiated metanephric mesenchyme (Table 3, Fig. 1B).

During the same developmental period, BMP-2/4 and BMP-7 positive cells have almost the same distribution pattern in all mesonephric structures (Table

Table 1. Age and number of human conceptuses analyzed.

Age (weeks)	Carnegie stage	No.
5-6	15-17	3
7	17-20	3
8-9	22- /	2

Table 2. Immunoreactivity to growth factor specific antibodies in the human mesonephros during the 5th to 9th week of development.

Antibodies/ Weeks of development	Wolffian duct	Mesonephric mesenchyme	Glomerule	Bowman's capsule	Mesonephric tubule	Coelomic epithelium
FGF-8						
5 and 6	+	+ +	+ +	+ +	+	+
7	+	+ +	+ +	+ +	+ +	+
8 and 9	+	+ +	+	+	+	+
FGF-10						
5 and 6	+ +	+ +	+ +	+	+	+
7	+	+	+	+	+ +	+ +
8 and 9	-	+ +	+	+	+	+
BMP-2/4						
5 and 6	+ +	+ +	+ +	+ +	+ +	+
7	+	+	+ +	+ +	+ +	+ +
8 and 9	-	+ +	+	+	+	+
BMP-7						
5 and 6	+	+ +	+ +	+ +	+	+
7	+	+	+ +	+	+ +	+ +
8 and 9	+	+ +	+	+	+ +	+
VEGF						
5 and 6	+ +	+	+	+	+ +	+
7	+	+ +	+	+	+ +	+
8 and 9	+ +	+	+ +	+ +	+ + +	+

+ + +, strong reactivity; + +, moderate reactivity; +, mild reactivity; -, no reactivity.

2). In the metanephros, moderate BMP-2/4 positivity is found in the ureteric bud and mesenchyme. In the ampulla and in the metanephric cup mitotic cells show moderate reactivity, while the other cells show mild reaction to BMP-2/4 (Table 3, Fig. 1C+). Only a few BMP-7 positive cells are detected in the ureteric bud, ampullae and in the metanephric cup, while the metanephric mesenchyme is characterized by very intense reactivity (Table 3, Fig. 1D).

In the mesonephros, moderate VEGF positivity is found in the Wolffian duct and in the mesonephric tubules, while other mesonephric structures show mild reactivity (Table 2, Fig. 1E). In the metanephros, moderate reactivity to VEGF is found in the ampullae, ureteric bud and metanephric cup, while mild VEGF reactivity characterizes the metanephric mesenchyme (Table 3, Fig. 1F).

During the 7th, 8th and 9th week of development, the mesonephros shows signs of degenerative changes only in its cranial parts. In the metanephros, the ureteric bud dilates and branches to form the collecting tubules ending with ampullae. The metanephric mesenchyme differentiates to form more metanephric cups, giving rise to renal vesicles, s-shaped nephrons and more mature nephrons containing renal corpuscles in medullary direction (Bowman's capsule and renal glomerulus). The rest of the metanephric mesenchyme forms interstitial connective tissue.

In the 7th developmental week, moderate FGF-8 reactivity is detected in most of the mesonephric structures. Moderate FGF-10 positivity is seen in the mesonephric tubules and in the coelomic epithelium, while all other structures express mild reactivity (Table 2). In the metanephros, moderate FGF-8 and FGF-10 reactivity is found in the collecting tubules and in the interstitium, while mild positivity is found in the metanephric cup, renal vesicles and s-shaped nephrons. No FGF-8 positive cells are seen in the ampulla (Table 4).

Table 3. Immunoreactivity to growth factor specific antibodies in human metanephros during the 5th to 6th week of development.

Antibodies	Ureteric bud	Ampulla	Metanephric mesenchyme	Metanephric cup
FGF-8	-	-	+	-
FGF-10	+ +	+	+ +	+
BMP-2/4	+ +	+	+ +	+
BMP-7	+	+	+ + +	+
VEGF	+ +	+ +	+	+ +

+ + +, strong reactivity; + +, moderate reactivity; +, mild reactivity; -, no reactivity.

During the 8th and 9th developmental week, FGF-8 and FGF-10 positive cells have the same distribution pattern in the mesonephros, with the exception of the Wolffian duct, which is devoid of FGF-10 positive cells (Table 2). All metanephric structures express moderate FGF-8 positivity, except for the interstitium, characterized by strong reactivity. Within those structures, some cells show clear reaction, while in others reaction is completely missing (Table 4, Fig. 1A) Moderate FGF-10 positivity is found in some cells of the interstitium and collecting tubules, while mild reactivity is present in the metanephric cup and in the developing nephrons, except for the mitotic cells which are clearly FGF-10 positive (Table 4, Fig. 2B).

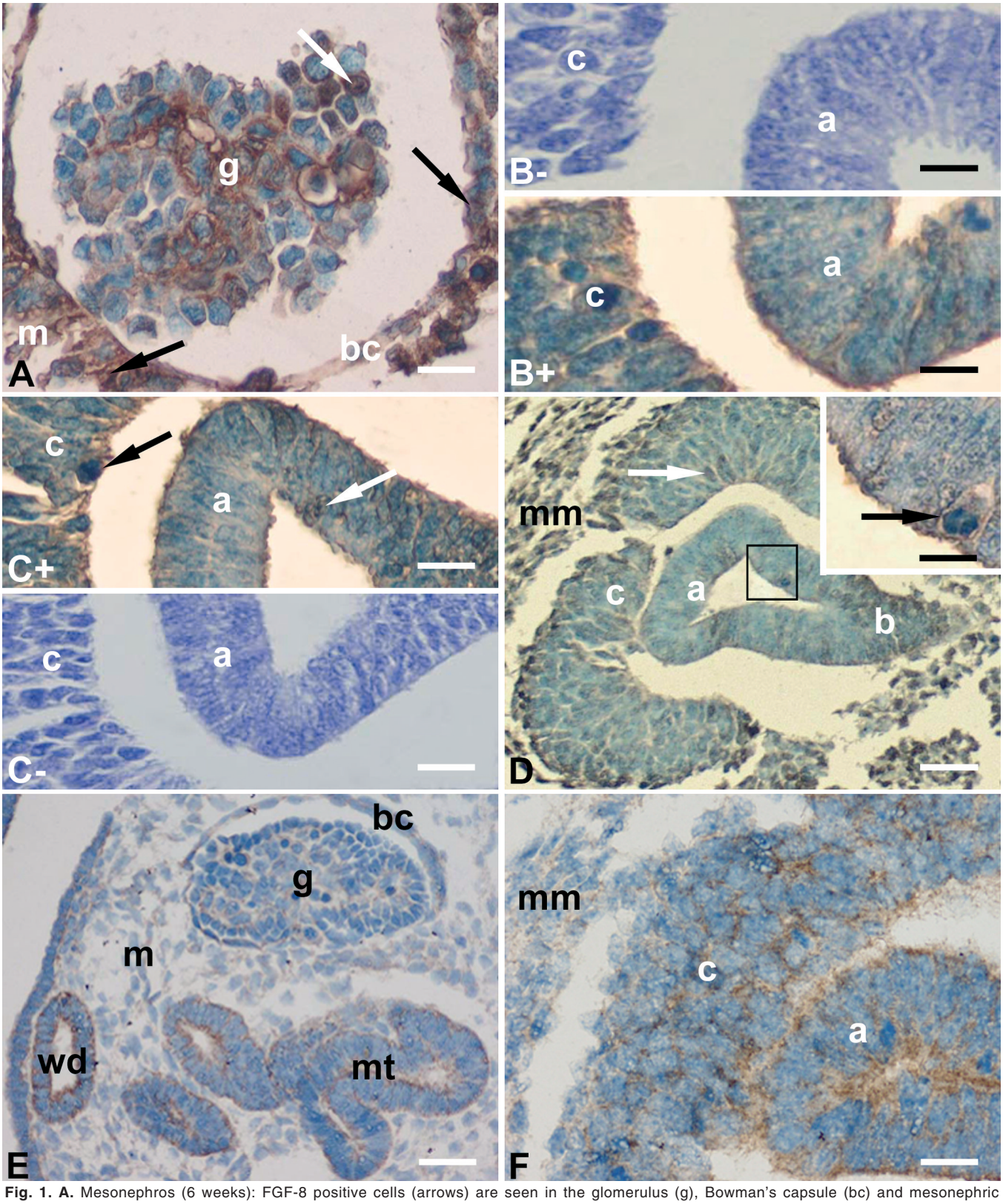

Fig. 1. A. Mesonephros (6 weeks): FGF-8 positive cells (arrows) are seen in the glomerulus (g), Bowman's capsule (bc) and mesonephric mesenchyme (m). Immunostaining to FGF-8, x 40. Scale bar 18 µm. **B+.** Metanephros (5 weeks): FGF-10 is positive in the metanephric cup (c) and ampulla (a). Immunostaining to FGF-10, x 40. Scale bar 10 µm. **B-.** Metanephros (5 weeks): Negative control to FGF-10 staining. **C+.** Metanephros (5 weeks): BMP-2/4 positivity (arrows) characterizes mitotic cells of the metanephric cup (c) and ampulla (a), while other cells show only mild reaction. Immunostaining to BMP-2/4, x 40. Scale bar 18 µm. **C-.** Metanephros (5 weeks): Negative controle to BMP-2/4 staining, x 40. Scale bar 18 µm. **D.** Metanephros (5 weeks): note strong reaction to BMP-7 antibody in the mesenchyme (mm), while only mitotic cells (arrow) in the metanephric cup (c) and ampulla (a) show mild reaction. Immunostaining to BMP-7, x 10. Scale bar 43 µm. Insert: mitotic cell (arrow) in the ampulla shows BMP-7 positivity. Immunostaining to BMP-7, x 100. Scale bar 13 µm. **E.** Mesonephros (6 weeks): VEGF positive cells characterize Wolffian duct (wd), mesonephric tubules (mt), glomerulus (g), Bowman's capsule (bc) and mesonephric mesenchyme (m). Immunostaining to VEGF, x 20. Scale bar 43 µm. **F.** Metanephros (5 weeks): VEGF moderately positive cells are seen in the ampulla (a) and metanephric cup (c), and mildly positive in the metanephric mesenchyme (mm). Immunostaining to VEGF, x 40. Scale bar 21 µm.

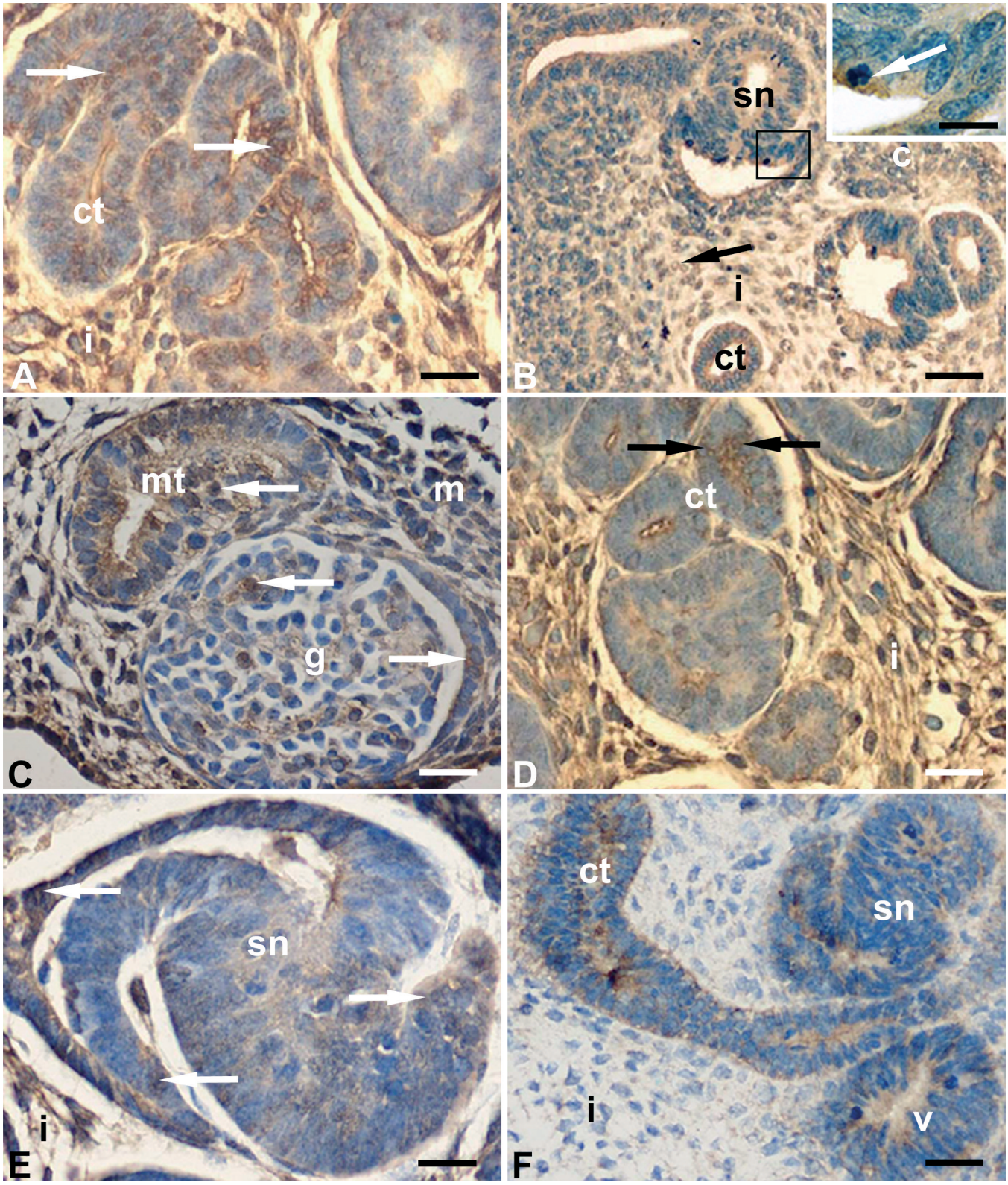

Fig. 2. A. Metanephros (9 weeks): some cells (arrows) in the collecting tubules (ct) show clear reaction to FGF-8, while stronger reaction is seen in the surrounding interstitium (i). Immunostaining to FGF-8, x 40. Scale bar 24 μm. **B.** Metanephros (8 weeks): note moderately FGF-10 positive cells (arrows) in the interstitium (i) and in the collecting tubules (ct). Some cells of the metanephric cup (c) and differentiating nephrons (sn) show clear FGF-10 positivity. Immunostaining to FGF-10, x 20. Scale bar 47 μm. Insert: Mitotic cell (arrow) of the differentiating nephron shows strong reaction to FGF-10. Immunostaining to FGF-10, x 100. Scale bar 14 μm. **C.** Mesonephros (9 weeks): some cells (arrows) of the glomerulus (g), mesonephric tubules (mt), and Bowman's capsule (bc) show clear BMP-7 reactivity, while all cells of the surrounding mesenchyme (m) are moderately positive to BMP-7. Immunostaining to BMP-7, x 40. Scale bar 20 μm. **D.** Metanephros (9 weeks): Some cells of the collecting tubules (ct) are characterized by clear BMP-2/4 reactivity (arrows), while surrounding interstitium (i) shows moderate reactivity. Immunostaining to BMP-2/4, x 40. Scale bar 24 μm. **E.** Metanephros (9 weeks): some cells of the s-shaped nephron (sn) show BMP-7 positivity (arrows), while interstitial cells (i) show stronger reaction. Immunostaining to BMP-7, x 40. Scale bar 15 μm. **F.** Metanephros (9 weeks): mild VEGF reaction is seen in the interstitium (i), renal vesicle (v) and s-shaped nephron (sn), while it is stronger in the collecting tubules (ct). Immunostaining to VEGF, x 20. Scale bar 29 μm.

Table 4. Immunoreactivity to growth factor specific antibodies in human metanephros during the 7th to 9th week of development

Antibodies/ Weeks of development	Collecting tubules	Ampulla	Interstitium	Metanephric cup	Renal vesicle	S-shaped nephrons	Renal corpuscle
FGF-8							
7	+ +	-	+ +	+	+	+	+
8 and 9	+ +	+ +	+ + +	+ +	+ +	+ +	+ +
FGF-10							
7	+ +	+	+ +	+	+	+	/
8 and 9	+ +	+	+ +	+	+	+	+
BMP-2/4							
7	+	+	+	+	+	/	/
8 and 9	+	+	+ +	+	+	+	+
BMP-7							
7	+ +	+ +	+	+	+	+ +	/
8and 9	+ +	+ +	+ +	+ +	+	+	+
VEGF							
7	+ +	+	+	+	+	/	/
8 and 9	+ +	+	+	+	+	+	+

+ + +, strong reactivity; + +, moderate reactivity; +, mild reactivity; -, no reactivity; /, structure absent in the tissue section.

During the 7th developmental week, both BMP-2/4 and BMP-7 positive cells are present in all mesonephric structures (Table 2). All metanephric structures are characterized by mild BMP-2/4 reactivity (Table 4). Moderate BMP-7 positivity is detected in the collecting tubules, ampulla and s-shaped nephrons, while mild reactivity is present in all other metanephric structures (Table 4).

Between the 8th and 9th week of development, BMP-2/4 and BMP-7 positive cells are present in all mesonephric structures and have the same distribution pattern, except for the Wolffian duct, which is devoid of BMP-2/4 positive cells (Table 2, Fig. 2C). During that developmental period, both factors are present in all the structures forming the metanephros (Table 4, Fig.2D), but now the BMP-7 expression has increased in the collecting system and in the metanephric cup (Table 4, Fig. 2E).

In the 7th developmental week, moderate VEGF positivity is present in the mesonephric mesenchyme and in the mesonephric tubules, while mild reactivity is found in all other structures (Table 2). In the metanephros, only the collecting ducts are characterized by moderate VEGF reactivity, while all other metanephric structures express mild VEGF positivity (Table 4).

In the period between 8th and 9th developmental week, VEGF reactivity has increased in all mesonephric structures, particularly in the mesonephric tubules (Table 2). Mild VEGF positivity is present in all metanephric structures except the collecting tubules, where VEGF shows moderate reaction (Table 4, Fig. 2F).

Discussion

The mesonephros

In our study, analysis of FGF-8 and FGF-10 distribution pattern showed cells positive to both factors in all human mesonephric structures. All mesonephric structures contained BMP-2/4 and BMP-7 positive cells as well. The decrease in reactivity of both growth factors and of BMP-2/4 subgroup detected in the early fetal mesonephros, might be considered as a sign of gradual regression of the transitional mesonephric kidney.

All mesonephric structures were characterized by VEGF expression, whose intensity increased in the early fetal period. Simon et al. (1995) previously reported the presence of VEGF in the glomerular visceral epithelial cells of the human fetal mesonephros. Our data on VEGF expression pattern in the mesonephros might indicate the involvement of VEGF in the formation of the mesonephric blood vessel network, as a previously suggested role for the metanephric vessel network formation (Kaipainen et al., 1993; Simon et al., 1995, 1998; Tufro et al., 1999; Tufro, 2000; Kim and Goligorsky, 2003).

The metanephros

During the early embryonic period (5-6 weeks) of human metanephric development, the FGF-8 positivity was restricted only to metanephric mesenchyme. At later stages, it appeared with increasing intensity both in the collecting system and in the developing nephrons. These results are in accordance with the data on mice FGF-8 mRNA localization in the metanephros (Grieshammer et al., 2005). Thus, the possible source of FGF-8 might be the developing nephrons. Disrupted nephrogenesis in FGF-8 mutant mice due to extensive apoptosis suggested that FGF-8 signaling might be required for cell survival. When applied to the human metanephric development, FGF-8 could influence nephron formation by controlling apoptosis. A similar developmental function was addressed to bcl-2 protein in the developing human kidneys (Carev et al., 2006). Thus, the decrease in FGF-

8 expression in the human kidney development might result in severely disrupted nephrogenesis and extensive apoptosis leading to kidney malformations at birth.

Contrary to FGF-8, FGF-10 reactivity was detected in all metanephric structures at early developmental stages, particularly in the mesenchyme. During further development, FGF-10 reactivity gradually decreased. The results of our study, together with the investigation on the rat ureteric bud (Qiao et al., 2001), might indicate that the interstitium and the autocrine FGF-10 secretion are the main source of FGF-10. Through stimulation of ureteric bud growth and branching, FGF-10 seems to control the number of induced nephrons. As also shown for mice (Ohuchi et al., 2000), FGF-10 deficiency during the described developmental period might lead to abnormalities in human kidney development, primarily to reduction of the number of nephrons. Besides importance of FGF-10 in the growth of the collecting system and nephrogenesis, FGF receptors might also be important for mesenchymal-epithelial cell interactions in the developing kidney (Celli et al., 1998).

In our study, the BMP-2/4 subgroup reactivity was described for the first time in the human metanephric tissue. It was more positive in the ureteric bud and mesenchyme than in the developing nephrons. Although we could only speculate about single BMP (BMP-2 or BMP-4) distribution pattern, the BMP-2/4 pattern of appearance that we found was much more ubiquitous than both single BMP (2 and 4) expression patterns together, reported in the previous study performed in the mouse by Dudley and Robertson (1997). In that study, BMP-2 mRNA expression was first restricted to the metanephric cup and later appeared transiently in the developing nephrons, while BMP-4 was found in the mesenchyme surrounding the developing collecting system and in the renal corpuscle. The possible source of BMP2/4 might be the metanephric mesenchyme and developing nephrons. Due to our results, the involvement of both factors in the collecting system development, as also suggested in the previous studies on mammalian metanephric development (Piscione et al., 1997, 2001; Miyazaki et al., 2000, 2003), might be applicable for the developing human kidney. Renal abnormalities, as well as the urinary tract abnormalities, reported in the several studies on BMP-4 heterozygous mice (Zhang and Bradley, 1996; Dunn et al., 1997; Miyazaki et al., 2000; Martinez et al., 2002), might occur because of disrupted BMP-4 expression during human permanent kidney development as well.

In our study, the BMP-7 expression was present in all metanephric structures from 5th to 9th developmental week, being strongest in the metanephric mesenchyme during early stages. At later stages, BMP-7 decreased in the developing nephrons, but increased in the collecting tubules. This time-sequence of appearance of BMP-7 protein coincided with localization and pattern of BMP-7 mRNA expression (Vukicevic et al., 1994). This led to the suggestion that BMP-7 was released from the developing glomerules and subsequently accumulated in the basement membranes of tubular epithelium (Vukicevic et al., 1994; Helder et al., 1995). As BMP-7 mRNA was found also in the ureteric bud, both the developing nephrons (tubules) and collecting system could be the source of BMP-7 protein. Our data, together with the results on BMP-7 expression in the mouse metanephric development (Luo et al., 1995; Dudley et al., 1995, 1999; Dudley and Robertson, 1997; Jena et al., 1997; Godin et al., 1999; Piscione et al., 1997, 2001), might indicate that BMP-7 signaling is required to enable continuous growth of the human metanephros (Schedl and Hastie, 2000) and that the deficiency in that signaling might lead to kidney abnormalities such as kidney dysplasia (Dudley et al., 1995). BMP-7 seems to have a crucial role in epithelial-mesenchymal conversion and induction of the metanephros, as well as in nephrogenic differentiation (Vukicevic et al., 1996).

During the investigated developmental period, all metanephric structures were characterized by VEGF expression, especially the ureteric bud, ampullae and the metanephric cup in the early embryonic period (5-6-weeks). At a later developmental period, the strongest expression was detected in the collecting ducts. VEGF expression in the early human metanephric development indicates that the early metanephric structures are already initiating blood vessel formation. The results of our study accord with the previous studies on VEGF and its receptors performed on human fetal metanephros (Kaipainen et al., 1993; Simon et al., 1995, 1998). All those studies indicate that VEGF is mitogenic for vascular endothelial cells (Kaipainen et al., 1993; Hyink and Abrahamson, 1995). Additionally, it promotes differentiation of the endothelial cells, but also the proliferation of tubular cells, as well as capillary network formation (Tufro et al., 1999). VEGF receptors were expressed predominantly in the glomerular capillaries, while VEGF protein was found in distal tubules and glomerular epithelial cells (Tufro et al., 1999; Kim and Goligorsky, 2003). In experimental animals, VEGF deficient embryos had impaired all steps of vascular development including vasculogenesis (in situ differentiation of blood vessels) and angiogenesis (sprouting from preexisting vessels) (Carmeliet et al., 1996). As shown by Kaipainen et al. (1993), expression of VEGF receptors in human fetuses was modulated in a dynamic manner during development. The source of VEGF could be glomerular epithelial cells, but also the developing nephrons and collecting ducts. Developmental changes in expression pattern for VEGF protein described in our study could be associated with subsequent development of blood vessels in the differentiating kidney.

In conclusion, all investigated growth factors were dynamically reacting in the developing human mesonephros in the manner that accorded with growth and gradual regression of the mesonephric kidney system. VEGF presence indicated developed blood vessels in the transient kidney tissue. In the developing permanent human kidney (metanephros), several growth

factors appeared in parallel during the early kidney development, which was characterized by formation and branching of the ureteric bud. Thus, appearance of FGF-10, BMP2/4 and BMP-7 proteins could be associated with ureteric bud stimulation and branching due to their role in prevention of apoptosis. At later developmental stages, an increase of FGF-8 and BMP-7 reactivity indicated their involvement in control of nephrogenesis by the same process. Changes in the described pattern of appearance of the investigated growth factors might be associated with different kidney abnormalities, such as small and dysplastic kidneys or cyst formation. VEGF expression in the metanephros suggested early initiation of blood vessels formation, its mitogenic role in endothelial and tubular cells, and its importance in all steps of vascular development. Thus, abnormal VEGF expression might lead to impaired formation of blood vessels in the human kidneys.

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