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Expression of hypoxia-inducible transcription factors in developing human and rat kidneys

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Early kidney development is associated with the coordinated branching of the renal tubular and vascular system and hypoxia has been proposed to be a major regulatory factor in this process. Under low oxygen levels, the hypoxia-inducible transcription factor (HIF) regulates the expression of genes involved in angiogenesis, erythropoiesis and glycolysis. To investigate the role of HIF in kidney development, we analyzed the temporal and spatial expression of the oxygen regulated HIF-1α and -2α subunits at different stages of rat and human kidney development. Using double-staining procedures, localization of the HIF target geneproducts vascular endothelial growth factor (VEGF) and endoglin was studied in relation to HIFα. In both species, we found marked nuclear expression of HIF-1α in medullary and cortical collecting ducts and in glomerular cells. In contrast, HIF-2α was expressed in interstitial and peritubular cells podocytes of the more mature glomeruli. After completion of glomerulogenesis and nephrogenesis, HIF-1α and -2α were no longer detectable. The HIF-target gene VEGF colocalized with HIF-1α protein in glomeruli and medullary collecting ducts. HIF-2α colocalized with the endothelium-associated angiogenic factor, endoglin. Both HIFα isoforms are activated in the developing kidney in a cell-specific and temporally controlled manner, indicating a regulatory role of oxygen tension in nephrogenesis. HIF-1α seems to be primarily involved in tubulogenesis and HIF-2α in renal vasculogenesis. Both isoforms are found in glomerulogenesis, potentially having synergistic effects.

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The process of nephrogenesis is coordinated by complex molecular and physiological mechanisms that are still largely unknown. The development of the mammalian kidneys starts at days E11/12 in mice and rats and at gestational week 4–5 in humans (Figure 1). The process of nephrogenesis is initiated by the ureteric bud, which branches dichotomously and forms the origin of the collecting duct system. At the tip of the ureteric buds, mesenchymal cells form aggregates that epithelialize and initiate glomerulogenesis in four consecutive stages: (1) the vesicle stage, (2) the comma stage, (3) the S-shaped stage and (4) the definitive glomerulus.[1] The S-shaped bodies fuse with the collecting duct to form the nephron. Completion of kidney development in mice and rats takes place at days 7–8 post partum (Figure 1, upper panel). In humans, kidney development is terminated at gestational weeks 32–36 (Figure 1, lower panel).

Nephrogenesis is accompanied by the growth and development of the renal vascular system. Because of a mismatch of oxygen demand and vascularization, local oxygen tension is presumably low in early developmental stages. Thus, regional hypoxia is believed to play a major regulatory role in tissue maturation, albeit the underlying molecular mechanisms are incompletely understood. In recent years, the *hypoxia-inducible factor* (*HIF*) has been identified as a transcription factor, which is in a key position to control gene expression under low oxygen tensions.[2–4] Regulation involves angiogenesis, erythropoiesis, glycolysis, vascular tone, pH homeostasis and cell survival decisions like proliferation and apoptosis.[2,3,5] HIF is a heterodimer consisting of an oxygen regulated α-subunit (HIF-1α or HIF-2α) and a constitutively expressed β-subunit (HIFβ, aryl hydrocarbon receptor nuclear translocator). In the presence of oxygen, two critical prolines of the HIFα chains are hydroxylated by prolyl hydroxylases, which require molecular oxygen as substrate. The hydroxylated prolyl residues are recognized by the von Hippel–Lindau protein as a component of an E3 ubiquitin ligase, which targets HIFα for proteasomal degradation. Under hypoxic conditions, HIF accumulates in the cell and induces transcription of target

genes.[3,4] Studies on HIFα and HIFβ knockout mice[6–9] highlighted the importance of HIF in embryonic development. Knockout of HIF-1α resulted in severe cardiovascular malformations, defects of the neural tube and embryonic lethality at day E11.[8] Studies on HIF-2α knockout mice yielded inconsistent results in terms of lethality and phenotype. Peng *et al.*[6] described severe vascular malformations and embryonic death at day E9.5,[6] whereas Tian *et al.*[10] reported an increased but incomplete embryonic lethality at

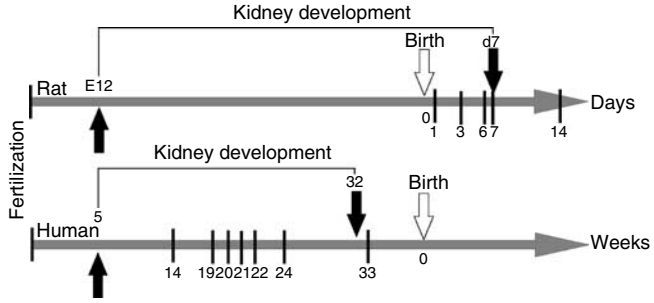

Figure 1 | Differential time course of rat and human kidney development. Renal development is initiated in early pregnancy as indicated by the black arrows (⬆). Human kidney development ends at the gestional week 32 (lower panel), whereas rat renal development continues post partum (| – examined time points, ⬇ – completion of kidney development, (▽) – birth).

Table 1 | Gestational week and fetal diseases of the examined human tissue

Week of gestation	Disease of the fetus
14	Uncharacterized chromosomal aberration
19	Trisomy 21
21	Complex malformations
22	Spina bifida
24	Bursted amnion
33	Dandy–Walker syndrome

Table 2 | Expression sites of HIF-1α and HIF-2α in developing rat and human kidneys compared to their target genes endoglin and VEGF

	HIF-1α	HIF-2α	VEGF	Endoglin
Human	CD medulla	Podocytes	CD medulla	Endothelial cells
	CD cortex S-shaped bodies Glomerular cells	Endothelial cells Interstitial cells (cortex, medulla)		
Rat	CD medulla	Podocytes	CD medulla	Endothelial cells
	CD cortex Glomerular cells	Endothelial cells Interstitial cells (medulla)		

CD, collecting ducts.

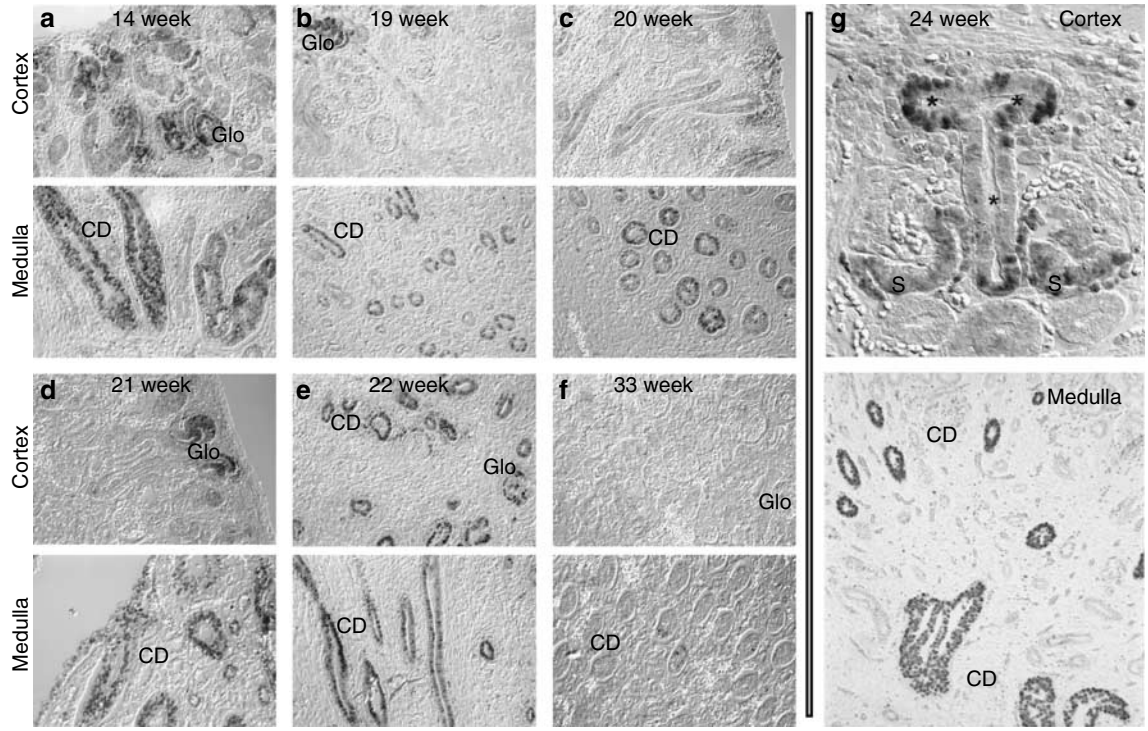

Figure 2 | HIF-1α expression in human kidney development. At the examined time points, HIF-1α was detectable from week 14 to 24 of human kidney development (**a-e**, **g**). After completion of renal development at week 33, signals for HIF-1α were almost absent (**f**). HIF-1α was strongly expressed in the nephrogenic zone in earlier stages of glomerular development (comma (**a**, **b**; cortex) and S-shaped bodies (**c-e, g**; cortex), whereas in the more mature glomeruli HIF-1α stained negative (**f**; cortex). In addition, medullary collecting ducts showed clear nuclear staining at every developmental stage (**a-e**, **g**; medulla). After completion of renal development, the collecting ducts were negative for HIF-1α (**f**, medulla). (**g**) Dividing collecting duct ampullae (*) with clear nuclear staining of HIF-1α. In direct vicinity epithelial cells of S-shapes (S) express HIF-1α as well (**g**). (CD – collecting ducts; Glo – glomerulus; Original magnification ×400; **g** cortex ×1000).