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FGF is essential for both condensation and mesenchymal–epithelial transition stages of pronephric kidney tubule development

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Abstract

The pronephros is a transient embryonic kidney that is essential for the survival of aquatic larvae. It is also absolutely critical for adult kidney development, as the pronephric derivative the wolffian duct forms the ductal system of the adult kidney and also triggers the condensation of metanephric mesenchyme into the adult nephrons. While exploring *Xenopus* pronephric patterning, we observed that epidermally delivered hedgehog completely suppresses pronephric kidney tubule development but does not effect development of the pronephric glomus, the equivalent of the mammalian glomerulus or corpuscle. This effect is not mediated by apoptosis. Microarray analysis of microdissected primordia identified FGF8 as one of the potential mediators of hedgehog action. Further investigation demonstrated that SU5402-sensitive FGF signaling plays a critical role in the very earliest stages of pronephric tubule development. Modulation of FGF8 activity using a morpholino has a later effect that blocks condensation of pronephric mesenchyme into the pronephric tubule. Together, these data show that FGF signaling plays a critical role at two stages of embryonic kidney development, one in the condensation of the pronephric primordium from the intermediate mesoderm and a second in the later epithelialization of this mesenchyme into the pronephric nephron. The data also show that in *Xenopus*, development of the glomus/glomerulus can be uncoupled from nephron formation via ectopic hedgehog expression and provides an experimental avenue for investigating glomerulogenesis in the complete absence of tubules.
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Introduction

Amphibian larvae utilize a simple embryonic kidney called the pronephros up until metamorphosis when it is supplanted by the permanent adult kidney. In essence, each pronephric kidney is a single giant nephron with a large external glomerulus or glomus (Field, 1891; Fox, 1963). In addition to being absolutely essential to the development and function of the adult kidney, the pronephros is also required for survival in amphibian and fish larvae, as bilateral pronephrectomy leads to rapid death due to edema (Howland, 1921). Histologically, the pronephric tubule has many of the same features as a mammalian cortical nephron such as a proximal tubule with a well-developed brush border and a columnar epithelia, followed by a distal tubule with a cuboidal epithelia and a duct with low cylindrical cells (Møbjerg et al., 2000; Vize et al., 2003a). Proximo-distal patterning of the pronephric nephron recapitulates that of the adult kidney nephrons of both amphibians and mammals with a segmented organization and at least six different functional domains specialized in specific ion transport processes (Zhou and Vize, 2004). Similar regulatory genes control development of both pronephroi and complex adult kidneys (Vize et al., 1997; Drummond, 2005) and genetic interactions identified in model systems such as *Xenopus* pronephroi (Carroll and Vize, 1999; McLaughlin et al., 2000) have subsequently been found to hold true in mammalian kidney development (Bouchard et al., 2002; Cheng et al., 2003).

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Pronephric development is initiated during gastrulation when inductive interactions between the anterior somites and lateral plate specify the pronephric mesoderm (Seufert et al., 1999; Mauch et al., 2000; James and Schultheiss, 2003). The anterior portion of the pronephros, both tubular and glomerular, is specified in *Xenopus* by Nieuwkoop and Faber (NF) stage 12.5 whereas the more posterior region is specified by stage 14. Once specified, presumptive pronephric tissue can be explanted and grown ex vivo in a basic saline solution and still differentiate into the appropriate kidney structures (Brennan et al., 1998, 1999). At around the time at which specification occurs, Pax8 (XPax-8, Carroll and Vize, 1999) and lim1 (Xlim-1; Taira et al., 1994) gene expression is activated in the presumptive kidney region. A few hours later, WT1 expression initiated in the presumptive pronephric glomus (xWT1, Carroll and Vize, 1996; Drummond et al., 1998; Semba et al., 1996). Some 8–9 h following the activation of early kidney specification genes, pronephric morphogenesis is initiated when the future pronephric nephron mesoderm segregates away from the lateral plate and somites on its borders and begins its posterior migration (Nieuwkoop and Faber, 1994; Vize et al., 2003b). The migrating mesenchymal pronephric duct fuses with the rectal diverticulum, an outgrowth of the cloaca, to complete the tubular portion of the pronephric system. While the duct is still migrating posteriorly, a wave of epithelialization moves along the primordium from anterior to posterior converting the condensate into a hollow tubule (Nieuwkoop and Faber, 1994). Approximately 12 h after the morphogenesis of the tubular portion of the pronephros begins, the external glomerulus of the pronephros, the glomus, pushes inwards into the coelom and is vascularized by the dorsal aorta (Gerth et al., 2005; Nieuwkoop and Faber, 1994). The glomus looks much like an enlarged version of a mammalian glomerulus with a mesangial matrix and well-developed podocytes (Drummond et al., 1998; Kluge and Fischer, 1990). It extends over multiple body segments and filters into the coelom rather than into a Bowman's capsule. From the coelom, the primary filtrate is swept into the proximal portion of the pronephric tubules through thin ciliated funnels known as nephrostomes (Vize et al., 1997, 2003b).

The above-described series of developmental and genetic process is largely recapitulated during mammalian nephron condensation. Rather than forming from the multipotential intermediate mesoderm as pronephroi do, mammalian nephrons condense from the metanephric blastema, a determined but undifferentiated pool of mesenchymal cells awaiting a passive inductive signal from the ureteric bud (Carroll and McMahon, 2003). Despite this potentially important difference, similar genes are associated with, and required for, both processes. As in pronephroi, key players include lim1, notch, Pax2, Pax8, wnt4, and WT1 (Carroll and McMahon, 2003; Jones, 2003).

In this report, the *Xenopus* system was used to explore the role of hedgehog signaling in pronephric patterning. The results indicate that, as previously proposed (Majumdar and Drummond, 1999), hedgehog (hh) itself plays no role in the establishment of the early kidney primordia. However, hh does possess a striking ectopic activity manifested on the developing kidney. When supplied to the pronephros from the overlying epidermis, hh completely suppresses pronephric tubule development while leaving glomeral development unaffected. This hh activity appears to function by ectopic regulation of essential kidney genes and can therefore be used to identify such genes. Microarray analysis of hh transcriptional targets indicates that suppression of FGF8 may contribute to the defects in pronephric primordium development. An essential role for FGF was confirmed through a combination of FGF signaling inhibitor studies and morpholino-mediated inhibition of FGF8 translation. FGF signaling is essential for maintenance of lim1, Pax8, and vHNF1 transcription and condensation of the pronephric primordium. FGF8 also functions during the mesenchymal to epithelial transition that converts the pronephric primordium into the epithelial tubules of the pronephric nephron and the pronephric/wolffian duct. Together these data demonstrate a critical requirement for FGF in pronephrogenesis and show that FGF8-independent glomerular development can occur in the complete absence of pronephric tubules.

Materials and methods

Nomenclature

The standard *Xenopus* nomenclature adopted by the NCBI for frog genes is utilized rather than the original gene names in order to maximize compatibility with data available from other model systems. Where possible *Xenopus* names are the same as the human ortholog.

Embryos, microsurgery, and in situ analysis

Xenopus laevis embryos were generated via standard techniques (Sive et al., 2000). Ectopic hh was supplied to the developing kidney by microinjecting the animal pole of 2-cell stage embryos with 100 pg of banded hedgehog mRNA (Ekker et al., 1995), either in one or both blastomeres.

In situ hybridization protocols for colorimetic staining were derived from Sive et al. (2000). Fluorescent in situs were performed according to Zhou and Vize (2004) and Gerth et al. (2005). Whole-mount TUNEL assays were performed on bleached embryos following the staining protocols of Hensey and Gautier (1998) and Veenstra et al. (1998).

Zebrafish embryos were obtained through timed matings of heterozygous carriers of the smu^{b641} allele of slow muscle omitted/smoothened. This allele changes a glycine to an arginine in the second transmembrane domain of the Smoothened protein (Varga et al., 2001). Embryos were raised at 28°C and staged according to Kimmel et al. (1995) before being fixed overnight at 4°C in 4% paraformaldehyde in PBS. RNA in situ hybridization was performed following Thisse et al. (1993). Digoxigenin or fluorescein-labeled antisense RNA probes were generated for pax2.1 and wt1 (Drummond et al., 1998) and detected using anti-digoxigenin or anti-fluorescein antibodies conjugated to alkaline phosphatase (Roche), followed by incubation with BM purple (Roche) or iodonitrotetrazolium chloride (INT; Sigma) and 5-bromo 4-choro 3-indolyl phosphate (Roche).

Morphant

A morpholino, FGF8-MO, 5′ CCAGGATGGAGGTGATGTAGTTCAT 3′ was generated (GeneTools) targeting both *X. laevis* FGF8A and FGF8B variants, based on a previously published reagent (FGF8-AS) that has been demonstrated to effectively block translation of both these mRNAs (Park et al., 2004). One presumptive pronephric blastomere, V2.2 (Moody, 1987), was injected with 14 ng of morpholino resuspended in 0.1× MR (Sive et al., 2000). FITC-dextran (FLDx; 70 kDa) was coinjected with the morpholino to trace injected blastomeres. When necessary, the tracer was detected using anti-FITC-POD (Roche) and either a red peroxidase substrate (Vector Laboratories) or a FITC-

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