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Stage specific requirement of Gfrα1 in the ureteric epithelium during kidney development

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ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) binds a coreceptor GDNF family receptor α1 (GFRα1) and forms a signaling complex with the receptor tyrosine kinase RET. GDNF-GFRα1-RET signaling activates cellular pathways that are required for normal induction of the ureteric bud (UB) from the Wolffian duct (WD). Failure of UB formation results in bilateral renal agenesis and perinatal lethality. Gfrα1 is expressed in both the epithelial and mesenchymal compartments of the developing kidney while Ret expression is specific to the epithelium. The biological importance of Gfrα1's wider tissue expression and its role in later kidney development are unclear. We discovered that conditional loss of *Gfrα1* in the WD epithelium prior to UB branching is sufficient to cause renal agenesis. This finding indicates that Gfrα1 expressed in the nonepithelial structures cannot compensate for this loss. To determine Gfrα1's role in branching morphogenesis after UB induction we used an inducible *Gfrα1*-specific Cre-deletor strain and deleted *Gfrα1* from the majority of UB tip cells post UB induction in vivo and in explant kidney cultures. We report that *Gfrα1* excision from the epithelia compartment after UB induction caused a modest reduction in branching morphogenesis. The loss of Gfrα1 from UB-tip cells resulted in reduced cell proliferation and decreased activated ERK (pERK). Further, cells without Gfrα1 expression are able to populate the branching UB tips. These findings delineate previously unclear biological roles of Gfrα1 in the urinary tract and demonstrate its cell-type and stage-specific requirements in kidney development.

1. Introduction

The development of the metanephric kidney, the permanent mammalian kidney, requires ureteric budding, reciprocal interactions between the ureteric bud (UB) epithelium and the metanephric mesenchyme (MM), branching morphogenesis and nephrogenesis. Defects in these processes can result in hypoplasia with low nephron endowment which is a risk factor for the development of hypertension and chronic kidney disease in adulthood (Abitbol and Ingelfinger, 2009). Global gene knockout studies have helped identify a number of genes that are important in kidney development (www.gudmap.org) (Costantini and Kopan, 2010; Dressler, 2009; Little et al., 2010). Further investigation of these developmental genes in a cell and time-specific manner are necessary to gain insights into how these genes and downstream pathways can be manipulated to alter the course of kidney diseases resulting from their deficiencies.

Glial cell line-derived neurotrophic factor (GDNF) coreceptor α1 (GFRα1) is a major protein that is essential for early UB induc-

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tion and kidney development as global *Gfrα1*-knockout mice die at birth due to renal agenesis (Cacalano et al., 1998; Enomoto et al., 1998). This has hampered further studies of the role of Gfrα1 in later renal development. GFRα1 is the high affinity co-receptor for GDNF. GFRα1 is anchored to the cell surface by glycosylphophatidyl inositol (GPI) linkage. It localizes to lipid rafts within the cell membrane and the binding of GDNF recruits the receptor tyrosine kinase rearranged during transfection (RET). Among the four GFRα coreceptors (GFRα1–4) and four GDNF family ligands (GFLs, GDNF, Neurteurin, Atremin and Persephin), the receptor complex consisting of dimers of GFRα1-GDNF with RET is the physiologically relevant signaling unit during kidney and enteric nervous system development (Saarma, 2000). This three protein complex of GDNF, GFRα1, and RET activates the phosphorylation of specific tyrosine residues in the intracellular domain of RET. This results in activation of downstream signaling pathways such as PLCγ, PI3K, and/or MAPK that have been shown to be necessary for early embryonic kidney development (Jain, 2009; Jain et al., 2006a).

Gfrα1 mRNA is expressed in both the ureteric epithelium and the MM during branching morphogenesis (Enomoto et al., 2004; Golden et al., 1999; Towers et al., 1998). Thus, it is more widely expressed than either Gdnf or Ret during kidney development. The biological significance of this wider and persistent expression after UB induction is not clear. A number of studies in vitro suggest that GFRα1/GDNF/RET tyrosine kinase signaling has a role in branching morphogenesis, a process that occurs after UB induction – reviewed in (Costantini and Shakya, 2006). However, evidence in vivo for the role of this pathway in later metanephric development is lacking. Another interesting aspect of Gfrα1 is that it can act in *trans* in neurological tissues (Ledda et al., 2002; Paratcha et al., 2001). The physiological role of trans-signaling by Gfrα1 in kidney development is not known.

Here we generated unique Gfrα1 reporter and Cre-deletor strains to conditionally examine Gfrα1's role in the epithelia cell in pre and post UB kidney development. We found that Gfrα1 was expressed in the mesonephric mesenchyme and Wolffian duct (WD) before UB induction and in the MM and UB epithelium during branching morphogenesis. Expression of Gfrα1 in the urinary epithelium is critical for UB induction and has a modest role after UB induction during early branching morphogenesis.

2. Results

2.1. *Gfrα1 is expressed early in kidney development and throughout branching morphogenesis*

To obtain a clear understanding of Gfrα1 expression in the urinary tract during development we generated a Gfrα1 reporter mouse that expresses the enhanced green fluorescent protein (EGFP) from the *Gfrα1* locus (*Gfrα1*$^{EGFP/+}$) (Uesaka et al., 2007) (Fig. 1A). Robust EGFP signal, indicative of Gfrα1 expression, was identified throughout the developing WD at E9.5 (Fig. 1B). At E10.5, Gfrα1 expression was identified in the WD with greater localization to the caudal UB budding domain, as well as in the mesonephric mesenchyme and the cranial mesonephric tubules (future epididymis in males) (Fig. 1C). At E11.5, the T-shape stage of branching morphogenesis, Gfrα1-positive cells were present in the UB stalk and in the distal UB tips (Fig. 1D and E). There was faint expression of Gfrα1 in the metanephric mesenchyme (MM) at E11.5 (Fig. 1E) that becomes more prominent as the MM condenses around the UB tip (Fig. 1F). At E13.5, consistent with previously reported mRNA expression of *Gfrα1*, we detected Gfrα1-positive cells in the "cap" mesenchyme in addition to the ureteric epithelium (Fig. 1G–I) (Enomoto et al., 2004; Golden et al., 1999; Towers et al., 1998). At E15.5 strong EGFP expression was seen in the developing kidney by whole mount immunohistochemistry (Fig. 1J). High resolution images (Fig. 1K and M) confirm both UB tip and cap mesenchyme expression. Thereafter, Gfrα1 continues to be expressed in the developing kidney both in the ureteric stalk (collecting duct), bud tip and the surrounding cap mesenchyme (Fig. 1N and O). We performed double label immunohistochemistry with anti-Gfrα1and anti-EGFP antibodies to confirm that EGFP expression recapitulated Gfrα1 expression (Fig. 1P–R). These observations support that Gfrα1 has a broader expression in the metanephric kidney than either Ret or Gdnf (Enomoto et al., 2004; Golden et al., 1999; Sainio et al., 1997; Towers et al., 1998) and demonstrate that Gfrα1 is expressed before UB induction in both the Wolffian Duct (WD) and the surrounding mesonephric mesenchyme.

2.2. *Gfrα1 expression in the collecting system primordia is indispensable for metanephric kidney formation*

Previous studies have demonstrated that forced expression of *Gfrα1* from the *Ret* locus while eliminating it from non-Ret expressing sites is sufficient for normal kidney development (Enomoto et al., 2004). This suggested that trans-Gfrα1 expression is dispensable if Gfrα1 is expressed in a cell-autonomous manner. However, non-epithelial expression of Gfrα1 in other organ systems has shown that it can act in a paracrine manner, so called "trans-signaling," to activate Ret signal transduction (Ledda et al., 2002; Paratcha et al., 2001). Therefore, we sought to determine if Gfrα1 from sites other than the urinary epithelium are able to compensate and support normal kidney development. We used the Cre-deletor strain Hoxb7-Cre (Yu et al., 2002) to achieve WD-specific deletion of *Gfrα1* in mice harboring a *Gfrα1*-conditional reporter allele *Gfrα1*^{floxEGFP} (Uesaka et al., 2007) (Fig. 2A). All *Gfrα1*$^{floxEGFP/-}$:*Hoxb7Cre* pups, which have *Gfrα1* deleted in the urinary tract epithelium, had kidney defects. The majority had bilateral renal agenesis/aplasia (16/20) and three had unilateral agenesis and contralateral megaureter (Fig. 2B and Table 1). These findings reveal unequivocally that Gfrα1 in the WD/urinary epithelium is indispensable and is the mechanism for renal agenesis in global *Gfrα1*-null mice. Further, trans-Gfrα1 in early stages of kidney development cannot compensate for its loss in the WD.

2.3. *Conditional deletion of Gfrα1 after initial UB budding using a tamoxifen inducible Cre strain*

After determining that Gfrα1 is required in the WD during early kidney development and that it is expressed throughout metanephric development we next established an inducible system to delete *Gfrα1* after UB induction. To accomplish this, we generated a mouse strain that expresses the tamoxifen