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ORIGINAL ARTICLE

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Fibroblast growth factor receptor signaling through MEK–ERK is required for prostate bud induction

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Abstract The urogenital sinus (UGS) is specified as prostate in mice around embryonic day 15.5 as indicated by expression of the transcription factor *Nkx3.1*. Shortly thereafter, growth of epithelial buds into the UGS mesenchyme initiates prostatic morphogenesis. A comparison of male and female UGSs *in vivo* demonstrated sexually dimorphic expression of branching morphogenesis regulatory genes coincident with epithelial budding including *Bmp7*, *Gli1*, *Gli2*, *Fgf10*, *Ptch1*, and *Shh*. A comparison of UGSs grown with or without testosterone in serum-free organ cultures showed that some, but not all sexually dimorphic differences observed during prostate bud induction, were effectively modeled *in vitro*. Organ cultures were then used to investigate the role of fibroblast growth factor receptor (FGFR) signaling during prostatic induction. Blocking FGFR activation with PD173074 showed that activation of extracellular signal-regulated kinase 1/2 (ERK1/2) in the UGS is dependent on FGFR signaling. Furthermore, inhibiting either FGFR activation with PD173074 or ERK1/2 activation with UO126 blocked all morphogenesis, proliferation, and gene expression changes induced by androgens in the UGS. These data reveal a previously unknown role for ERK1/2 during prostate bud induction. They also show that signaling by FGFRs through ERK1/2 is required for androgen-induced budding morphogenesis, proliferation, and gene expression during prostate bud induction.

Key words androgen · MapK · ERK1/2 · fibroblast growth factor receptor · urogenital sinus · prostate · FGF10 · branching morphogenesis · FGFR2 · testosterone

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Introduction

Female and male urogenital sinus (UGS) development diverges shortly after the fetal testes begin production of testosterone (Marker et al., 2003). In males, testosterone specifies the UGS as prostate while in females the UGS develops into part of the vagina. The first known molecular change during prostate specification in the male rodent UGS is transcriptional up-regulation of *Nkx3.1* around embryonic day 15.5 (e15.5) (Bhatia-Gaur et al., 1999). At this stage in development, the androgen receptor is localized to the urogenital sinus mesenchyme (UGM), suggesting a paracrine mesenchyme-to-epithelial signal for prostatic induction (Cooke et al., 1991). At approximately e17, prostatic morphogenesis commences with the budding of the urogenital sinus epithelium (UGE) into the UGM (Hayward et al., 1996). The epithelial buds elongate, bifurcate, and finally differentiate to form the complex ductal structure called the prostate gland (Marker et al., 2003). Androgens are necessary and sufficient to specify the UGS as prostate. This is shown by the absence of a prostate in Tfm mice that lack functional androgen receptors, and by the induction of a prostate in female UGSs treated with androgens (Cunha, 1975; Takeda et al., 1986; Brown et al., 1988; He et al., 1994; Donjacour et al., 2003). Experiments utilizing recombinant grafts of UGE and UGM from wild type and Tfm mice demonstrated the requirement for androgen receptor expression in the UGM, and not the UGE for prostatic bud induction and branching morphogenesis (Cunha and Lung, 1978; Donjacour and Cunha, 1993). This has led to the theory that one or more factors act in a paracrine fashion to stimulate prostatic development. The hypothesized androgen-regulated paracrine factor(s)

have been referred to as andromedin(s). An andromedin must fulfill three criteria: (1) it must be regulated directly or indirectly by androgens, and its absence must result in the loss of the prostate formation, (2) it has to be secreted from the UGM in the same temporal pattern as prostatic induction, and (3) it must be sufficient to induce prostatic development (Donjacour et al., 2003). The precise mechanism by which the andromedin functions to induce prostate development is unknown, and it could be acting as a positive regulator of prostate development or acting as a suppressor of one or more prostate antagonists. Alternatively, multiple andromedins could act in a combinatorial fashion to induce prostate formation. Although there have been many candidate andromedins, to date no single protein has been identified that mediates all androgen-induced changes during early prostate development.

Two proteins that have been evaluated as candidate andromedins are fibroblast growth factor 7 [FGF7; (Yan et al., 1992; Sugimura et al., 1996; Lu et al., 1999; Thomson and Cunha, 1999; Donjacour et al., 2003)] and FGF10 (Fasciana et al., 1996; Thomson et al., 1997; Thomson, 2001; Donjacour et al., 2003). *Fgf7* is globally expressed in the mesenchymal compartment of many tissues including the prostate; however, its expression levels *in vivo* are inversely correlated with androgen levels (Thomson et al., 1997). Furthermore, *Fgf7* knock-out mice were not reported to have abnormalities in the prostate (Guo et al., 1996). FGF10 has a more spatially restricted expression pattern in the UGM, surrounding active budding areas in the UGS, and some reports show that androgens can induce expression of *Fgf10* (Lu et al., 1999; Thomson and Cunha, 1999). However, other reports suggest that androgen cannot induce *Fgf10* (Thomson and Cunha, 1999). Furthermore, *Fgf10* is expressed in both male and female UGSs (Thomson and Cunha, 1999). Consequently, there is an ongoing controversy whether or not *Fgf10* is androgen regulated, but its role in prostatic morphogenesis is undeniable. *Fgf10* knock-out animals, which die at birth, do not initiate prostatic development. Postnatal development of the UGS from *Fgf10* knock-out mice has been examined experimentally by grafting knock-out UGSs into host mice and by *in vitro* organ cultures of knock-out UGSs (Donjacour et al., 2003). Grafts of knock-out UGSs had very low incidence of prostatic branching and differentiation. Treatment of cultured UGSs with exogenous FGF10 alone could not induce prostatic bud induction, but treatment of cultured UGSs with exogenous FGF10 and testosterone could only partially restore prostatic bud induction, suggesting that FGF10 knock-out mice have defects in the UGS that precede prostatic induction. In addition, FGF10 was not capable of inducing the UGS to form a prostate in the absence of testosterone (Donjacour et al., 2003). These experiments together with the results of treating prostatic epithelial cells with recombinant FGF10 have suggested that FGF10 acts as a pro-proliferative signal for the UGE during prostate specification (Lu et al., 1999).

An alternative theory to the single andromedin is the smooth muscle theory. This suggests that testosterone blocks an increase in the thickness of the smooth muscle layer separating the UGE from sources of constitutive paracrine signals in the UGM, thereby permitting paracrine signaling (Thomson et al., 2002). In summary, although several models and candidate andromedins have been proposed, existing data have not defined a single molecular intermediate between the activation of androgen receptor in the UGM and the induction of prostatic identity in the UGE.

Although little is known about the mechanism of prostatic induction, the FGF family, transforming growth factor β (TGFβ) superfamily, and Sonic hedgehog (SHH) pathways have been implicated as regulators of branching morphogenesis after prostatic induction (Podlasek et al., 1999; Thomson and Cunha, 1999; Lamm et al., 2001). The role of FGF receptor 2 (FGFR2), a receptor tyrosine kinase activated by FGF7 and FGF10, in branching morphogenesis has thus far only been implied. FGFR2 has two alternatively spliced isoforms that have been extensively studied in the literature, FGFR2(IIIb) and FGFR2(IIIc), and in the prostate FGFR2(IIIb) is the predominant alternatively spliced isoform present (Hughes, 1997). Both isoforms have different ligand-binding specificities and have different spatial localization patterns. Throughout the body, FGFR2(IIIb) is epithelially expressed and FGFR2(IIIc) is mesenchymally expressed (Orr-Urtreger et al., 1993; Yan et al., 1993; Finch et al., 1995; Ornitz et al., 1996; Feng et al., 1997). Several *Fgfr2* mutant alleles have been engineered in mice. Null alleles result in early embryonic lethality (Arman et al., 1998). Other alleles that eliminate the function of specific *Fgfr2* isoforms or eliminate *Fgfr2* in a tissue-specific manner have shown that *Fgfr2* is essential for the development of several organs including bone (Yu et al., 2003), lung (Peters et al., 1994; Arman et al., 1999), skin (De Moerlooze et al., 2000), and limbs (Xu et al., 1998; Revest et al., 2001).

Receptor tyrosine kinases can activate a variety of signaling cascades, some of which have been associated with the branching process, including several of the mitogen-activated protein kinase (MapK) cascades. One MapK cascade includes activation of Map/Erk kinase 1 (MEK1) and MEK2 (hereafter referred to as MEK1/2), which in turn phosphorylate extracellular signal-regulated kinase 1 (ERK1) and ERK2 (hereafter referred to as ERK1/2). ERK1/2 phosphorylate multiple substrate proteins that can regulate gene expression and other cellular processes (Gille et al., 1992; Davis, 1993; Janknecht et al., 1993). Genetic interaction studies in *Drosophila* demonstrate that MEK1/2–ERK1/2 is indispensable for proper branching morphogenesis during