Prostatic growth and development are regulated by FGF10

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SUMMARY

We have examined the role of Fibroblast Growth Factor 10 (FGF10) during the growth and development of the rat ventral prostate (VP) and seminal vesicle (SV). FGF10 transcripts were abundant at the earliest stages of organ formation and during neonatal organ growth, but were low or absent in growth-quiescent adult organs. In both the VP and SV, FGF10 transcripts were expressed only in a subset of mesenchymal cells and in a pattern consistent with a role as a paracrine epithelial regulator. In the neonatal VP, FGF10 mRNA was expressed initially in mesenchymal cells peripheral to the peri-urethral mesenchyme and distal to the elongating prostatic epithelial buds. At later stages, mesenchymal cells surrounding the epithelial buds also expressed FGF10 transcripts. During induction of the SV, FGF10 mRNA was present in mesenchyme surrounding the lower Wolffian ducts and, at later stages, FGF10 transcripts became restricted to mesenchymal cells subadjacent to the serosa. We investigated whether the FGF10 gene might be regulated by androgens by analysing the levels of FGF10 transcripts in SV and VP organs grown in serum-free organ culture. While FGF10 transcript levels increased after treatment with testosterone in the SV (but not VP), these changes were not sensitive to anti-androgen treatment, and thus it is likely that FGF10 mRNA was not directly regulated by testosterone. Also, FGF10 mRNA was observed in the embryonic female reproductive tract in a position analogous to that of the ventral prostate in males suggesting that FGF10 is not regulated by androgens in vivo. Recombinant FGF10 protein specifically stimulated growth of Dunning epithelial and BPH1 prostatic epithelial cell lines, but had no effect on growth of Dunning stromal cells or primary SV mesenchyme. Furthermore, FGF10 protein stimulated the development of ventral prostate and seminal vesicle organ rudiments in serum-free organ culture. When both FGF10 and testosterone were added to organs in vitro, there was no synergistic induction of development. Additionally, development induced by FGF10 was not inhibited by the addition of the anti-androgen Cyproterone Acetate demonstrating that the effects of FGF10 were not mediated by the androgen receptor. Taken together, our experiments suggest that FGF10 functions as a mesenchymal paracrine regulator of epithelial growth in the prostate and seminal vesicle and that the FGF10 gene is not regulated by androgens.

Key words: Fibroblast Growth Factor 10, FGF10, Prostate, Seminal vesicle, Testosterone, Androgen, Rat

Abbreviations: SV, Seminal vesicle; VP, ventral prostate; FGF10, Fibroblast growth factor 10; KGF, Keratinocyte growth factor; FGFR2, Fibroblast growth factor receptor 2; SVM, Seminal vesicle mesenchyme; VMP, Ventral mesenchymal pad.

INTRODUCTION

The development of the male reproductive tract is dependent upon androgens and mesenchymal-epithelial interactions. Androgens masculinise the reproductive tract during the latter third of gestation, and are required for the formation of the male sex accessory organs including the prostate and seminal vesicle (Cunha et al., 1987). Studies using tissue recombination have shown that androgen receptor action in mesenchymal cells is essential for prostatic induction, and that mesenchymal androgen receptors are both necessary and sufficient for prostatic ductal development (Cunha and Chung, 1981). This has led to the hypothesis that paracrine factors, which are produced by the mesenchyme and regulated by androgens, control the development of the male reproductive tract. Genes that are known to be regulated by androgens are generally secretory proteins or other molecules involved in the differentiated function of prostate epithelial cells. The identity of androgen-regulated mesenchymal paracrine acting factors involved in the development of the male reproductive organs is unknown. While several growth factors are known to be expressed in the prostate only two are thought to function as mesenchymal paracrine acting factors; these are Keratinocyte growth factor (KGF, FGF7) and Hepatocyte growth factor (HGF; Story, 1995). KGF and HGF are not restricted to the prostate (or hormone target organs) and are unlikely to act as mediators of androgen action, although both play important roles in growth regulation throughout organs of the body. It remains to be seen if prostate-specific growth factors exist; it is possible that some growth factors may show partial
restriction to the prostate or restriction to hormone target organs.

HGF functions as a regulator of epithelial morphogenesis in several organs but has not been extensively studied in the prostate. In contrast, there has been a significant amount of work on the function of KGF in the prostate. KGF was shown to play a key role in the development of the seminal vesicle (SV; Alarid et al., 1994) and ventral prostate (VP; Sugimura et al., 1996), and functioned as a mesenchymal paracrine regulator of epithelial growth and branching morphogenesis. In vitro, KGF was shown to be androgen-regulated (Yan et al., 1992) and thus KGF was proposed to be a mediator of androgen action (Peel and Rubin, 1995). However, KGF transcripts do not appear to be directly androgen regulated in vivo (Thomson et al., 1997). Deletion of KGF by gene knock-out had a surprisingly mild phenotype (Guo et al., 1996) and no effect upon the structure or function of the male reproductive tract was reported. Consequently, KGF does not appear to be a direct mediator of androgen action. Clearly, KGF does not show prostate-specific expression, although it does play a key role in epithelial growth and establishes an important role for the Fgf family in regulation of prostatic ductal growth and development. KGF is expressed in most of the organs systems in the body during development and therefore may play a general role in growth regulation (Finch et al., 1995; Mason et al., 1994), and the mild phenotype of the KGF null mouse may be due to redundancy within the Fgf family. This led us to hypothesise that perhaps KGF could mimic other members of the Fgf family that showed more restricted patterns of expression, and may be limited to a few organs including the prostate. Another member of the Fgf family, FGF10 (Yamasaki et al., 1996), has been shown to have a high degree of homology with KGF and to share many biochemical similarities (Igarashi et al., 1998). FGF10 is expressed in fewer organs than KGF and also shows a more restricted pattern of expression within tissues. FGF10 is typically expressed in mesenchymal cells that are associated with areas of active epithelial growth, in contrast to KGF, which is uniformly and diffusely expressed throughout mesenchymal tissues (Finch et al., 1995; Mason et al., 1994).

FGF10 was identified in a screen for molecules with homology to the Fgf family in the lung (Yamasaki et al., 1996). It was shown to be expressed in the adult lung and during development of the mouse embryo. Recent work has shown that FGF10 functions as a paracrine regulator of lung epithelia during development (Bellusci et al., 1997; Park et al., 1998). In the lung, FGF10 was expressed in mesenchyme surrounding growing epithelial lung buds and was mitogenic for epithelial but not mesenchymal cells (Bellusci et al., 1997). FGF10 has also been shown to play a key role in limb induction, where it appears to function as a primary limb inducer produced by the mesoderm (Ohuchi et al., 1997; Xu et al., 1998). Thus FGF10 acts as a paracrine regulator in several developing organs. Recently, deletion of the FGF10 gene resulted in loss of limbs and lungs, confirming the importance of FGF10 function during induction of these organs (Min et al., 1998). The status of the male reproductive tract development has not yet been reported in the FGF10 null mice, but since these animals survive until birth it will be possible to examine the effects of FGF10 deletion upon urogenital development.

FGF10 and KGF bind to Fgf receptor 2 (FGFR2) to transduce their effects (Igarashi et al., 1998). Deletion of the FGFR2 by gene knock-out has demonstrated a requirement for FGFR2 in placentation and limb development, and resulted in death of homozygous FGFR2 mutants between day 10 and 12 of embryogenesis (e10-12; Xu et al., 1998). The effect of FGFR2 deletion on limb development was consistent with studies suggesting an important role for FGFR10 in limb induction (Ohuchi et al., 1997). No effects of FGFR2 deletion were observed on lung development. Lung development starts at e9.5, and FGFR2 mutants die at e10-12, which corresponds with the initiation of bronchial branching morphogenesis. Inhibition of FGFR2 signalling in lung epithelia using a dominant negative FGFR2 receptor has shown that it is required for lung branching (Peters et al., 1994). Since development of the male sex accessory organs occurs at the end of gestation (e17 onwards), it will not be possible to examine the effect of this FGFR2 deletion on urogenital development.

We have examined the role of FGF10 in the androgen-dependent development of the prostate and seminal vesicle. Our data suggest that FGF10 functions as a key paracrine regulator of growth and development of these organs.

MATERIALS AND METHODS

FGF10 sequences

FGF10 sequences were cloned by RT PCR using primers complementary to the rat FGF10 mRNA sequence (accession number D79215; Yamasaki et al., 1996). Primer sequences were as follows: 5' primer, 5'-GAAAAGAACCAGGCAAGTC-3'; 3' primer, 5'-TGAACGGAAGGGTTTTTC-3'. RT PCR was performed as previously described using total RNA from neonatal VP and SV (Haughney et al., 1998). Conditions were one cycle of PCR (annealing at 56°C) followed by 29 cycles with 53°C annealing for 30 seconds, 72°C extension for 30 seconds and denaturation for 30 seconds at 94°C. The primers used generated a 323 nt band which was excised from a 2% agarose gel and subcloned into the PCRII vector (Invitrogen, Carlsbad, California).

RNA analysis

Total RNA was prepared from organs, cells or tissue samples using RNA STAT 60 (Tel Test 'B'; Freindswood, Texas) according to the manufacturers instructions. Antisense riboprobes for RNase protection and in situ hybridisation were prepared using T7, T3 and SP6 polymerases and 32P- or 35P-labelled nucleotides (NEN, Boston, Massachusetts). RNase protection assays were performed as described in detail in Thomson et al. (1997). Sizes of protected products in the RNase protection assay were rFGF10 323 nucleotides, cyclophilin 103 nucleotides and were sized by comparison to Century RNA markers (Ambion, Austin, Texas).

In situ hybridisation

In situ hybridisation was performed essentially as previously described (Frohman et al., 1990). Paraffin-imbedded sections (5 μm thickness) were de waxed, rehydrated and refixed in 4% paraformaldehyde for 20 minutes. Slides were treated with pronase (20 μg/ml) for 10 minutes, followed by treatment with acetic anhydride (diluted 1:300) in 0.1 M triethanolamine (twice for 5 minutes). Slides were then dehydrated through ethanol and hybridised with sense or anti-sense 35P-labelled riboprobes in humidified chambers. Sections were incubated with 6x106 cts/minute of probe per slide in 100 μl of hybridisation buffer (50% deionized formamide, 0.3 M NaCl, 20 mM Tris-HCl pH 8.0, 5 mM EDTA, 10
mM NaPO₄ pH 8.0, 10% dextran sulfate, 0.5 mg/ml yeast tRNA) overnight at 60°C under parafilm coverslips. Slides were washed in 5x SSC for 10 minutes (55°C) to remove parafilm coverslips, followed by 30 minutes in 2x SSC, 0.01 M DTT, 50% formamide at 55°C. Slides were washed in 0.5 M NaCl, 10 mM Tris pH 8.0, 0.1 mM EDTA pH 8.0 at 37°C for 10 minutes three times followed by incubation in the same buffer with RNase A (20 µg/ml) for 30 minutes at 37°C. Slides were washed in 2x SSC, 0.01 M DTT, 50% formamide at 55°C for 30 minutes followed by 2x SSC and 0.2x SSC at 37°C for 15 minutes each. Slides were dehydrated through graded ethanol and air dried. To localise specifically bound riboprobe, slides were dipped in Kodak NTB3 film emulsion and exposed for 3 weeks. Slides were developed according to the manufacturer’s instructions.

Sections were examined with a Leica DM RB microscope using dark-field illumination and images were captured with a Dage MTI DC 330 digital camera (Michigan City, Indiana) using Scion Image software (Frederick, Maryland). Dark-field images were manipulated with Adobe Photoshop software, such that the silver grain image was inverted, the silver grains were selected and coloured red, and superimposed upon a light-field image of the specimen. Micrographs shown are all antisense probe, there was no labelling of sections with sense probe.

**Cell and organ culture**

Neonatal SVs and VPs were microdissected from 0-day-old F344 or Wistar rats and grown in serum-free organ culture. Organs were treated with testosterone and KGF as previously described (Alarid et al., 1994; Sugimura et al., 1996), while recombinant FGF10 (kindly supplied by Amgen Inc., Thousand Oaks, California) was added to organ cultures at 200 ng/ml.

BPH1 (Hayward et al., 1995) and DU145 cells (Yan et al., 1993) were passaged in RPMI1640 with 5%FBS and plated out on 4-well glass slides for growth assays. Growth assays were performed in RPMI1640 with 1% charcoal-stripped FBS (DCC FBS) with addition of recombinant FGF10 protein. DT3 prostate stromal cells and primary rat seminal vesicle mesenchyme (SVM) were passaged in 50:50 DMEM:Hams F12 with 10% FBS, growth assays were performed in the same medium but using only 1% DCC FBS with the addition of recombinant FGF10. Epithelial or stromal cells were grown for 2, 4, 6 and 8 days with 10, 50, 200 ng/ml of FGF10, or with no additions. After culture, cells were fixed and stained with hematoxylin, and images were captured with a Dage MTI DC 330 digital camera (Michigan City, Indiana). Cells were counted using Scion Image software (Frederick, Maryland). Cell counts were expressed as percentage of control and three independent experiments were averaged.

**RESULTS**

The expression of FGF10 mRNA in neonatal rat organs was examined by RNase protection assay, shown in Fig. 1. FGF10 transcripts were present at high levels in the lung, epididymis, seminal vesicle and ventral prostate, while lower levels were observed in the skin (Fig. 1A). FGF10 transcripts were absent in the liver and heart, while the neonatal rat testis and kidney expressed very low levels of FGF10 transcripts. It was interesting to note relatively abundant expression of FGF10 transcripts in the epididymis and SV, since these organs exhibit a coiled or folded morphology and do not contain branched structures. Next, the ontogeny of FGF10 mRNA in the SV and VP was examined to identify changes in FGF10 expression between neonatal and adult periods. While FGF10 mRNA levels were high in the neonatal period (2 day), there was a progressive decrease in 10-day-old samples and at puberty (20 days old), while FGF10 mRNA was very low or absent during adult life (Fig. 1B). The decrease in FGF10 mRNA levels was most pronounced in the VP compared to the SV.

To examine the distribution of FGF10 transcripts, in situ hybridisation was used to localise FGF10 mRNA in histological sections of neonatal SV and VP at stages when FGF10 transcripts were shown to be abundant. In both the SV and VP, FGF10 expression was restricted to mesenchymal cells, while FGF10 mRNA was not detected in epithelia (Fig. 2). In VP of newborn (0-day-old) rats, expression of FGF10 transcripts was restricted to a subset of mesenchymal cells peripheral to the peri-urethral mesenchyme (Fig. 2A). In 2-day-old VP, FGF10 mRNA was highest in mesenchyme at the periphery of the organ, surrounding growing ductal tips which were undergoing branching morphogenesis (Fig. 2B). Thus, in the VP, FGF10 mRNA was localised in mesenchyme distal to the urethra and was most abundant in mesenchyme surrounding rapidly growing epithelial ducts (Sugimura et al., 1986). Next, we examined FGF10 mRNA distribution during the development of the SV. In embryonic day 18 embryos, FGF10 transcripts were present in mesenchyme surrounding the lower Wolffian ducts where SVs will develop (Fig. 2C). In neonatal SVs, FGF10 mRNA was expressed throughout the mesenchyme (Fig. 2D), in contrast to the pattern in the VP. While the epithelial ducts of the VP have been shown to grow along a proximodistal axis, with maximal DNA synthesis in epithelial tips distal from the urethra (Sugimura et al., 1986), the SV has not been reported to exhibit such a growth pattern. Therefore, the pattern of FGF10 transcript expression

![Fig. 1. Expression pattern and ontogeny of FGF10 transcripts. FGF10 mRNA was quantitated by RNase protection assay in various neonatal organs (A) and at different stages of SV and VP development (B). Total RNA from neonatal (2-day-old) lung, liver, heart, skin, testis, kidney, epididymis, seminal vesicle (SV) and ventral prostate (VP) was analysed for FGF10 mRNA expression. (B) The ontogeny of FGF10 expression was examined in SV and VP RNA from 2-day-old, 10-day-old, 20-day-old and adult animals.](image-url)
correlated well with the growth pattern of the VP, and may also correlate with the different growth pattern of the SV.

Since FGF10 mRNA was present during the earliest periods of SV organogenesis, we examined similar stages of VP development. In embryonic day 20 male urogenital sinus, FGF10 transcripts were detected in the ventral mesenchymal pad (VMP; Fig. 3). The VMP is a condensed mass of mesenchymal cells peripheral to the urethra and the peri-urethral mesenchyme, and were absent from epithelial cells. (B) A transverse section through a 2-day-old VP, the urethra (not shown) is at the bottom of the micrograph. FGF10 mRNA was present in mesenchyme surrounding epithelial buds at the tips of the branched VP epithelium, and not in mesenchyme proximal to the urethra at the base of the epithelial ducts. (C) A transverse section of an embryonic day 18 urogenital sinus at the lower end of the Wolffian ducts (labelled*) in the area where the seminal vesicles will develop. FGF10 transcripts were present in the mesenchyme surrounding the Wolffian ducts (WDM) and were absent in the ductal epithelia (WDE). (D) A longitudinal section through a 2-day-old SV. FGF10 mRNA was restricted to mesenchyme (SVM) subadjacent to the serosa (periphery) of the organ and was present homogeneously throughout mesenchyme along the proximodistal axis of the SV.

FGF10 transcripts were detected in the ventral mesenchymal pad (VMP; Fig. 3). The VMP is a condensed mass of mesenchymal cells at the periphery of the UGM, which has been shown to induce prostatic growth and branching morphogenesis in the presence of testosterone (Timms et al., 1995). Epithelial buds from the urethra grow towards the VMP and form the VP. Peri-urethral mesenchyme did not express FGF10 transcripts, but the VMP did (Fig. 3A). Other areas of the urogenital mesenchyme (UGM) also exhibited low levels of FGF10 mRNA, such as areas overlying the position where...
the dorsal prostate will form. Fig. 3D shows expression of FGF10 transcripts in the VMP in cross section. In this section, buds of the ventral prostate are visible above the VMP and will ramify into the VMP during VP organogenesis. At the top of the section, the tips of the developing SVs are present, one of which shows abundant FGF10 transcripts. FGF10 mRNA was also expressed in mesenchyme at the tips of the developing lateral prostate and in mesenchyme surrounding the epithelia of the anterior prostate (also known as the coagulating gland, Fig. 3C). Surprisingly, FGF10 expression was also observed in the VMP of embryonic day 20 female embryos (Fig. 3B). The VMP has been shown to be present in female embryos and can induce prostatic development in the presence of testosterone (Timms et al., 1995).

Next, we examined whether androgens might regulate FGF10 mRNA levels in organs grown in vitro (shown in Fig. 4). As controls, RNA from prostate epithelial cell lines NRP152 and Dunning Epithelial cell lines were included. These did not express FGF10 mRNA, but the Dunning prostate stromal cell line did express FGF10 transcripts, consistent with the in situ data indicating mesenchymal-specific expression of FGF10 mRNA. Neonatal SVs and VPs were grown in organ culture using serum-free medium for 5 days, in the presence of testosterone, KGF, or with no additions. In the absence of testosterone, there was little organ development, while treatment with testosterone or KGF induced significant growth and ductal branching morphogenesis and/or epithelial folding (Alarid et al., 1994; Sugimura et al., 1996). After culture, total RNA was prepared and FGF10 mRNA analysed by RNase protection assay and quantitated using a phosphoimager. In SVs there was a 4-fold increase in FGF10 mRNA after culture in the presence of testosterone, relative to untreated organs (Fig. 4A). However, when SVs were grown in the presence of KGF, FGF10 mRNA levels did not change compared to untreated organs. Under similar experimental conditions, KGF mRNA in SVs was previously shown to decrease in response to testosterone (Thomson et al., 1997). FGF10 mRNA levels in VPs grown in the presence of testosterone increased 1.5-fold relative to untreated or KGF-treated VPs (Fig. 4A). VPs treated with KGF or grown without any additions showed similar levels of FGF10 transcripts.

We further analysed the regulation of FGF10 mRNA by growing organs in the presence of testosterone for 4 days and then substituting the anti-androgen Cyproterone Acetate (CA) for 24 hours (Fig. 4B). We reasoned that, if testosterone directly upregulated FGF10 mRNA then treatment with the anti-androgen CA might reverse this effect. In the SV, FGF10 mRNA levels were elevated 4-fold after growth for 5 days in the presence of testosterone. However, SVs treated with testosterone for 4 days followed by CA for 24 hours did not show a reduction in FGF10 mRNA levels. A similar pattern was observed in the VP, although FGF10 transcripts only increased 1.5 fold after treatment with testosterone, and additional treatment with CA did not reverse this effect. It is important to note that these experiments measured steady-state transcript levels and that the elevated level of FGF10 mRNA following treatment with testosterone/CA may be due to a long FGF10 transcript half-life, and thus these experiments must be cautiously interpreted.

The paracrine nature of FGF10 signalling was established by addition of recombinant FGF10 protein to cultures of stromal or epithelial cells. Recombinant FGF10 was added to the prostate epithelial cell lines BPH1 and Dunning epithelial cells as well as primary cultures of seminal vesicle fibroblasts or the Dunning stromal cell line (Fig. 5). Recombinant FGF10 was added at 10, 50 and 200 ng/ml (or omitted), and cells were grown for 8 days. Results are shown as percentage of control cell growth. FGF10 stimulated growth of both BPH1 epithelial cells as well as Dunning epithelial cells, but did not affect either primary SV fibroblasts or Dunning stromal cells. The growth-stimulatory effect of FGF10 appeared to show some dose dependence since FGF10 added at 10 ng/ml resulted in approximately a doubling of cell number, while adding 200 ng/ml FGF10 resulted in approximately 4-fold more cells in both epithelial cell lines after culture for 6 days. The apparent decrease in cell number at the 8 day time point was due to FGF10-treated cells reaching high density and becoming confluent in some areas.

Next, we examined the effect of FGF10 on the development of VP and SV in serum-free organ culture in vitro. In the presence of testosterone or KGF both the VP and SV will undergo ductal branching morphogenesis and development.
Addition of recombinant FGF10 also induced significant epithelial morphogenesis in both VP and SV, after growth in vitro for 6 days (Fig. 6, third panels from left). In the VP, addition of FGF10 induced considerable branching morphogenesis, while in the SV the effect upon epithelial morphogenesis was less substantial. To examine if FGF10 and testosterone might act synergistically they were added simultaneously to cultures of SV and VP (Fig. 6, right panels). Addition of both FGF10 and testosterone induced growth similar to that induced by testosterone alone, and no synergism was observed under the culture conditions used here. To examine further if the testosterone and FGF10 pathways might interact, organs were grown in the presence of FGF10 and the anti-androgen CA (Fig. 7). Treatment with CA alone had no effect upon development of VP and SV relative to untreated organs (compare Fig. 6 left panels with Fig. 7 left panels) (Sugimura et al., 1996; Thomson et al., 1997), while treatment with CA and FGF10 resulted in some development which was similar to that induced by FGF10 alone. Thus, CA was not able to abolish FGF10-induced growth, in contrast to the observation that CA could inhibit KGF-induced growth (Sugimura et al., 1996; Thomson et al., 1997).

**DISCUSSION**

The pattern of FGF10 mRNA distribution in developing rat tissues indicated that FGF10 was expressed at high levels in organs of the foetal and neonatal male reproductive tract. Previous studies of FGF10 mRNA distribution have highlighted the expression of transcripts in branched organs such as the lung (Bellusci et al., 1997; Yamasaki et al., 1996). We have extended this finding to branched ductal organs of the male reproductive tract. Also, we observed FGF10 mRNA in epididymis and SV, which are organs that contain highly coiled or folded and not branched epithelia. Thus, FGF10 may play an important role in organs containing complex epithelial morphogenesis, including both branched and folded architecture. We have not observed significant levels of FGF10 transcripts at any stage (embryonic day 14 to 10 day postnatal) in the kidney (H.-T. Nguyen and A. A. T., unpublished observations), which has been widely used to study branching morphogenesis. Consequently, FGF10 is not present in all branched organs and instead may be a general regulator of complex epithelial morphogenesis.

The expression of FGF10 transcripts in the SV and VP was highest during the neonatal period and was substantially reduced or absent in adult organs. Both the SV and VP undergo considerable growth and development in the neonatal period, between birth and puberty when androgen levels are low (Donjacour and Cunha, 1988; Lung and Cunha, 1981). FGF10 transcripts were highest in these organs during neonatal growth.
FGF10 in prostate

and were significantly lower in adult organs, which are almost growth-quiescent. Thus FGF10 transcripts were most abundant during neonatal growth, when circulating androgen levels were low, which calls into question testosterone regulation of the FGF10 gene in vivo (see below for further discussion). KGF mRNA was also shown to be abundant during periods of SV and VP growth, and did not appear to be androgen regulated in vitro or in vivo (Thomson et al., 1997). The expression of FGF10 and KGF transcripts in the SV and VP only during periods of growth suggests that these Fgfs are involved in regulating epithelial growth of these organs, but may not be directly regulated by androgens.

Using in situ hybridisation, FGF10 transcripts were observed to be restricted to mesenchymal cells in both the SV and VP; consistent with the mesenchymal expression pattern of FGF10 in the developing lung (Bellusci et al., 1997). In the VP, FGF10 mRNA was found in mesenchyme peripheral to the peri-urethral mesenchyme, surrounding distal prostatic ductal tips undergoing branching morphogenesis. Analysis of epithelial cells in distal prostatic buds has shown that DNA synthesis is elevated relative to epithelial cells of proximal ducts near the urethra (Sugimura et al., 1986). Mesenchyme surrounding these growing prostatic ductal tips contained transcripts for FGF10, while mesenchyme surrounding urethral epithelium did not contain FGF10 mRNA. In the SV, FGF10 mRNA was also mesenchymal, but did not show a similar proximodistal distribution to that seen in the VP. Instead, FGF10 transcripts were evenly distributed along the proximodistal axis of the SV. The SV does not grow preferentially from its apical tip, and therefore exhibits a fundamentally different pattern of growth than the VP, even though both organs are dependent upon testosterone for their development. FGF10 was expressed in the SV and VP in a pattern consistent with that of a mesenchymal paracrine regulator of epithelium and consistent with differences in the pattern of epithelial growth of these organs.

FGF10 expression was analysed in developing urogenital sinus, to determine whether it might be involved in the induction of the SV and VP, since FGF10 appeared to function in the development of these organs. FGF10 mRNA was present in the mesenchyme surrounding the lower Wolffian ducts, which eventually form the seminal vesicles. It was also present in the VMP, a condensed mass of mesenchyme below the urethra, which induces the ventral prostate (Timms et al., 1995). Thus, FGF10 was present at the earliest stages of development of SV and VP, and may function as an important element of the induction of these organs as well as their subsequent growth and development. Intriguingly, FGF10 mRNA was also observed in the VMP of developing female embryos, which may be important when considering the regulation of FGF10 and possible mechanisms of prostate induction (see below). The FGFR2 (iiib) mRNA is expressed in all epithelial cells of the UGS in both males and females during embryonic and neonatal development (A. A. T., data not shown).

Recombinant FGF10 was able to stimulate growth of prostate epithelial cell lines, but did not stimulate growth of prostate stromal cells. Furthermore, recombinant FGF10 was able to stimulate the growth of SV and VP explants when cultured in vitro in a serum-free organ culture system in the absence of androgens. Therefore, we propose that FGF10 functions as a paracrine regulator of epithelial cell growth in both SV and VP development. Our studies have demonstrated that FGF10 has similar properties to KGF (Alarid et al., 1994; Sugimura et al., 1996), even though there are significant differences in the expression pattern of these two molecules. While FGF10 transcripts are expressed only in a subset of mesenchymal cells associated with rapidly dividing epithelial cells, KGF mRNA is expressed uniformly throughout the mesenchyme (Finch et al., 1995; Mason et al., 1994). This dramatic difference in expression pattern between FGF10 and KGF transcripts may have important consequences for the function of these molecules in vivo. Future studies must examine the distribution of FGF10 and KGF proteins. Igarishi et al. (1998) have shown that, although FGF10 and KGF have similar biochemical properties, there may be differences in their association with extracellular matrix. Similarities of sequence and function could result in functional redundancy between FGF10 and KGF, which may explain the extremely mild phenotype of KGF null mice (Guo et al., 1996). In contrast, FGF10 null mice show a severe phenotype and have no limbs or lungs (Min et al., 1998). Since KGF and FGF10 are both expressed in the lung, this suggests that KGF cannot completely replace FGF10 function, as KGF might have been expected to compensate for the lack of FGF10.

KGF and FGF10 have been shown to play important roles in lung growth and development (Bellusci et al., 1997; Post et al., 1996). Bellusci et al. (1997) showed that FGF10 induced branching of endoderm explants, while KGF induced a cystic-like growth but no branching. Recent studies using Fgf-soaked bead implants in lung rudiments have also shown differences in the biological activity of FGF10 and KGF (Park et al., 1998). FGF10 appeared toshow a chemotactic response in the lung epithelia whereas KGF did not, even though KGF induced mitogenesis. Both KGF and FGF10 signal through the same receptor (FGFR2iiib; Igarishi et al., 1998). This suggests that there may be differences in the biological functions of these molecules and in the intracellular signals generated by FGF10 and KGF.

A popular hypothesis is that the development of the male reproductive organs is controlled by mesenchymal paracrine acting factors that are regulated by androgens, and many studies have focussed on the identification of molecules that are induced by androgens. KGF was suggested to be a potential

Fig. 7. Effect of CA on FGF10-induced development. SV and VP organ rudiments were grown in the presence of the anti-androgen CA (left) or with CA and FGF10 (right). Scale bar, 1 mm.
mediator of androgen action in vitro (Peehl and Rubin, 1995; Yan et al., 1992), however, KGF transcripts did not appear to be regulated by androgens in vivo and therefore it is unlikely that KGF is a mediator of androgen action (Thomson et al., 1997). Similarly, FGF10 has recently been proposed to be a mediator of androgen action since it appeared to be regulated by androgens in vitro (Lu et al., 1999). However, data presented here suggest that FGF10 is not regulated by androgens in vivo. Our results showed that FGF10 transcript levels correlated inversely with androgen levels in vivo and that, in organs grown in vitro, androgen-induced changes in FGF10 transcripts could not be reversed by treatment with an anti-androgen. There was no regulation of FGF10 mRNA in primary cultures of prostate fibroblasts treated with testosterone (A. A. T., data not shown). Furthermore, FGF10 transcripts were observed in the female VMP, i.e. in the absence of androgens (Timms et al., 1995). Taken together it is evident that FGF10 transcript expression is probably not androgen-regulated in vivo even though prostate development is androgen-dependent. The VMP is able to induce prostatic development, but only in the presence of androgens after grafting in vivo. Females usually do not develop a prostate even though an FGF10-positive VMP forms. It is possible that FGF10 activity is regulated by androgens at the post-transcriptional level, and that androgens may affect the distribution or biological activity of FGF10. Extracellular matrix proteins, heparan sulfate or proteolytic enzymes may all be involved in regulating Fgf signalling, and it is possible that androgens may affect any of these regulatory steps. Indeed, evidence for the mesenchymal nature of androgen regulation of prostatic induction does not distinguish between induction of mesenchymal factors or their constitutive production but androgenic regulation of their activity. Thus it is possible that prostatic inducing molecules are made in female embryos, but are not active, due to a lack of correct distribution or biological activation.

To determine if testosterone and FGF10 might act synergistically, they were added together to organ cultures of SV and VP. There did not appear to be any synergism under the conditions used here, though it is possible that the development observed under androgenic stimulation is maximal in our system. We have also examined the ability of testosterone and FGF10 to synergise in prostatic bud induction in cultures of embryonic male and female UGS, but again have not observed any synergism between the two molecules (A. A. T., data not shown). The effect of the anti-androgen CA on FGF10-induced development was examined, and CA did not appear to inhibit FGF10-induced development. Since FGF10-induced growth is not inhibited by an anti-androgen, it suggests that the FGF10 is not acting via the androgen receptor (Culig et al., 1994). This is in contrast to KGF-stimulated development, which can be antagonised with CA (Sugimura et al., 1996; Thomson et al., 1997), suggesting that KGF-induced development may have been due to KGF acting through the androgen receptor. However, it is also possible that KGF-induced development was due to KGF mimicking FGF10, but since KGF-induced development was sensitive to CA antagonism (and FGF10 was not) this seems unlikely. The different properties of KGF and FGF10, with regard to CA antagonism, suggest that they may have different functions despite signalling through the same receptor. This is in agreement with the observations of KGF and FGF10 action in the lung and in KGF- and FGF10-null mice.

We have shown that FGF10 was able to function as a mesenchymal paracrine acting factor that regulates epithelial development in the prostate and seminal vesicle. Recombinant FGF10 stimulated growth of prostate epithelial cell lines but did not induce growth of a prostate stromal cell line or primary prostate fibroblasts. FGF10 was also able to stimulate the development of the VP and SV in organ culture and induced epithelial development similar to that induced by testosterone although, in the SV, FGF10-induced development was not as extensive as that induced by testosterone. FGF10 mRNA was present at the earliest periods of prostate and SV development and may function in both the induction as well as the growth and morphogenesis of these organs. FGF10 mRNA did not appear to be directly regulated by androgens in intact organs grown in vitro. Also, there was an inverse correlation of FGF10 transcripts with androgen levels in vivo, as well as expression of FGF10 mRNA in female VMP, which strongly suggested that the FGF10 gene is not directly androgen-regulated. Thus, further investigation of FGF10 regulation at the post-transcriptional level will be important.

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