FGF signalling in prostate development, tissue homoeostasis and tumorigenesis

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Synopsis

The FGFs (fibroblast growth factors) regulate a broad spectrum of biological activities by activating transmembrane FGFR (FGF receptor) tyrosine kinases and their coupled intracellular signalling pathways. In the prostate, the mesenchymal–epithelial interactions mediated by androgen signalling and paracrine factors are essential for gland organogenesis, homoeostasis and tumorigenesis. FGFs mediate these mesenchymal–epithelial interactions in the prostate by paracrine crosstalk through a diverse set of ligands and receptors. Gain- and loss-of-function studies in mouse models have demonstrated the requirement for the FGF signalling axis in prostate development and homoeostasis. The aberrant induction of this axis in either compartment of the prostate results in developmental disorders, disrupts the homoeostatic balance and leads to prostate carcinogenesis. FGFs are also implicated in mediating androgen signalling in the prostate between mesenchymal and epithelial compartments. Therefore, studying FGF signalling in the prostate will help us to better understand the underlying molecular mechanisms by which the gland develops, maintains homoeostasis and undergoes carcinogenesis; as well as yield clues on how androgens mediate these processes and how advanced-tumour prostate cells escape strict androgen regulations.

Key words: development, fibroblast growth factor (FGF), fibroblast growth factor receptor (FGFR), prostate, tissue homoeostasis, tumorigenesis

INTRODUCTION

The FGF (fibroblast growth factor) signalling family controls a broad spectrum of cellular processes throughout development, as well as in adult tissue homoeostasis, function and disease [1–3]. In humans, the prostate is a small acorn-shaped gland located at the base of the bladder, surrounding the urethra, and functions by secreting proteins that contribute to seminal fluid. Prostate cancer is the most common form of cancer and the second leading cause of cancer-related death in American men, with an estimated incidence of 192,000 (29% of all cancers in men) and mortality of 27,000 (9% of all male cancer deaths) in 2009. More than 2 million American men are currently living with prostate cancer. Androgen function and the reciprocal interactions between mesenchymal and epithelial compartments in the prostate are critical for normal gland morphogenesis and tissue homoeostasis. Disruption of this signalling can contribute to carcinogenesis and disease progression. The FGF family has long been implicated as one of the key paracrine mediators between prostate mesenchymal and epithelial compartments [4–6]. Gain- and loss-of-function mutations in mouse models have shed further light on the requirement for FGF signalling in prostate development and tumorigenesis [7–12]. Since many developmental events, including cell proliferation and differentiation, are recapitulated during tumorigenesis, studying prostate development will aid our understanding of prostate cancer generation. This review focuses on the current understandings of how FGFs and their receptors regulate prostate development and homoeostasis, as well as how aberrant FGF signalling can lead to prostate carcinogenesis.

THE FGF SIGNALLING SYSTEM

To date, 22 genes of the FGF family have been identified. These genes contain three exons and share high sequence homology within a central core domain that has been identified in both humans and mice [1,3]. FGFs are secreted from producing cells via autocrine, paracrine or endocrine loops to control a wide spectrum of cellular processes, including cell proliferation, differentiation, migration, survival and apoptosis [3,13,14].
FGFs exert their biological effects by binding to and activating transmembrane tyrosine kinase receptors (FGFRs) in concert with HSPGs (heparan sulfate proteoglycans). HSPGs are linear biopolymers with a high charge density and enormous combinatorial structural heterogeneity [15]. A large body of evidence demonstrates that heparan sulfates are required for FGFs to specifically bind and effectively activate certain FGFRs [16–19]. The FGFRs are encoded by four highly conserved genes defined as \( Fgfr1–Fgfr4 \), thus exhibit a variety of splice variants [2]. Each FGFR contains an extracellular ligand-binding domain, a single transmembrane domain and an intracellular domain with tyrosine kinase activity. The extracellular domain contains two or three Ig-like domains that are required for FGF binding, as well as an acidic region and a heparin-binding region which are located between the first and second Ig-like domains [14,20,21]. Among \( Fgfr1–Fgfr3 \), alternative splicing encompassing the C-terminal half of the third Ig domain generates either the IIIb or IIIc isoforms (Figure 1). This splicing is tissue-specific and affects the ligand-binding specificity, thereby increasing the functional diversity of FGFRs [18,22]. The intracellular domain is composed of a docking-protein-binding site, tyrosine kinase domains and a short C-terminal region.

Binding of FGF ligands to the FGFR–HSPG complexes enables dimerization of the receptor intracellular domains and induces autophosphorylation of the intracellular tyrosine kinase domain. This complex then further phosphorylates the adaptor protein FRS2 (FGFR substrate 2) [1,23]. The phosphorylated tyrosine residues in the FGFR also activate the PLC\( \gamma \) (phospholipase \( \gamma \)) pathway [24,25]; whereas phosphorylated FRS2 functions as a docking site to recruit alternative adaptor proteins, specifically Grb-2/Sos (growth-factor-receptor-bound protein 2/Son of sevenless), which activates the MAPK (mitogen-activated protein kinase) signalling cascades and the PI3K (phosphoinositide 3-kinase) pathway [23,26]. These signalling pathways activate downstream target proteins, including transcription factors in the nucleus (Figure 2).

The expression of FGFs and FGFRs is spatiotemporally-specific in embryos and tissue- and cell-type-specific in adults. Analyses of tissue expression patterns and phenotypes in mouse models of targeted gene disruption indicate that FGF signalling plays a critical role in multi-stage embryonic development and adult tissue homoeostasis [3]. Aberrant activation of FGF signalling pathways is found in developmental disorders and diverse adult-tissue-specific pathologies, including malignant cancer [8–12,27].

**OVERVIEW OF PROSTATE DEVELOPMENT AND TISSUE HOMEOEOSTASIS**

The prostate is a branched ductal gland located at the base of the bladder. The structures of the normal human and rodent prostate are distinct. In humans, the prostate is an acorn-shaped structure without lobular organization, characterized by three morphological regions: the peripheral zone, transitional zone and central zone [28]. In mice and rats, the prostate consists of eight distinct lobes in four directions, namely, the anterior, ventral, dorsal and lateral prostate. The dorsal and lateral lobes are considered counterparts of the human peripheral zone. At maturity, prostate ducts are composed of epithelial and stromal compartments, which are separated by a basement membrane. The epithelial compartment, including tall columnar epithelial cells, basal cells and rare neuroendocrine cells, is separated by the stromal compartment, which is composed of smooth muscle cells, fibroblasts and myofibroblasts [29]. The main function of the adult prostate is to secrete specific alkaline proteins that constitute 10–30% of the volume of the seminal fluid.

Mouse prostate development initiates at E17 (embryonic day 17) when a subset of UGS (urogenital sinus) epithelial cells derived from the hindgut endoderm grow out into the surrounding
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Figure 2  FGF signalling complex
Left-hand panel: extracellular domains of FGFRs form inactive dimers associated with HSPG. Right-hand panel: the binding of FGF ligands activates the receptor dimers and induces the phosphorylation of intracellular tyrosine kinases and docking protein FRS2, which then further activates downstream signalling pathways, including PI3K/AKT, PLCγ, and ERK1/2. II and III, the second and third Ig loops of the extracellular domains; F, FGFs; Grb2, growth-factor-receptor-bound protein 2; MEK, MAPK/ERK kinase; PKC, protein kinase C; SHP2, Src homology 2 domain-containing protein tyrosine phosphatase 2; sos, Son of sevenless.

UGS mesenchyme in distinct directions to form the anterior, ventral, dorsal and lateral prostate buds. These buds subsequently form the different prostate lobes, undergo extensive branching morphogenesis, elongate from distal points and form intraductal mucosal infolding (see Figure 4A). Approx. 80% of this ducatal branching is complete by day 10 of neonatal life and the whole process is complete within 2–3 months [30].

The normal development, function and tissue homoeostasis of the prostate are governed by androgens, which function through the interaction of mesenchymal and epithelial compartments [4,30–33]. At early developmental stages, androgens are required for the initiation of prostate development and for its embryonic and neonatal growth. During adult life, androgens maintain the production of prostate secretory proteins and prostate tissue architecture [4]. When androgens are lacking, the prostate atrophies due to apoptosis of terminally differentiated cells whose viability is dependent on these hormones. Approx. 35% of the ducatal tips and branch-points are lost in distal regions within 2 weeks after orchiectomy. Re-introduction of androgen induces active cellular proliferation in the epithelium of the atrophied prostate within 2 days, and the epithelial ducts completely regenerate within 14 days [34].

Androgenic effects on the prostate are mediated through the AR (androgen receptor), a member of the nuclear receptor transcription factor superfamily which is expressed in both stromal and epithelial cells. Upon binding of androgens in the cytoplasm, the AR translocates into the nucleus to bind to AREs (androgen-responsive elements) in the promoter region of targeted genes [35]. It has been proposed that paracrine growth factors between the stromal and epithelial compartments mediate the regulatory functions of androgens and are crucial for androgens to instruct epithelial cells undergoing proliferation and differentiation [32]. Reciprocal communication between the epithelia and mesenchyme may also play similar roles in stroma development, particularly in the differentiation to smooth muscle cells (Figure 3) [30,36]. Although the paracrine mediators of androgen action are not well understood, the FGF signalling axis has been implicated in this process.

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In the prostate, members of the FGF family and alternative splice variants of FGFRs are partitioned in the epithelium and
mesenchyme (stroma), thereby mediating directional and reciprocal communication between these two compartments. Both FGF7 and FGF10 mRNA are exclusively expressed by the mesenchyme, whereas their receptors are found in the epithelium of the prostate (Figure 3) [6]. Radial receptor-binding assays and covalent cross-linking analysis has revealed that FGF7 and FGF10 have equal affinity for the resident epithelial cell receptor FGFR2IIIb, but FGF-7 also binds the IIIb splice variant of FGFR1 [5]. Both FGF7 and FGF10 can specifically stimulate epithelial cell growth, but not stromal cell growth [5,6,37]. In addition, FGF7 and FGF10 can also substitute for androgens in organ culture of neonatal prostates, and support extensive epithelial growth and ductal-branching morphogenesis [6,38]. However, Fgf7-null mice have no visible defects in the male reproductive tract [39]; this may result from compensation by FGF10. Ablation of Fgfr10 alleles abrogates prostate development and diminishes androgen responsiveness of prostatic rudiments in organ-culture and tissue-recombination experiments [9]. Consistent with this effect, loss of Fgfr2 alleles in mouse prostatic epithelial precursor cells prevented development of the anterior and ventral prostate lobes beyond the initiation stage (E17.5) and disrupted tissue development and androgen responsiveness in the dorsolateral prostates [8] (Figure 4B). These ligand- and receptor-deleted mouse models strongly demonstrate the essential role of the FGF10/FGF2 signalling axis in prostate development and homeostasis.

Another reciprocal FGF signalling axis regulating prostate development and homeostasis is FGF9/FGFR3 (Figure 3). FGF9 mRNA is expressed exclusively in the epithelial cells, whereas FGFR3 is expressed at functionally significant levels only in the stromal cells [36]. Relative mitogenic activity assay and covalent affinity cross-linking assays suggest that FGF9 activates the FGFR2 and FGFR3 IIIc isoforms to levels of high activity [36,40], Although its function in prostate development remains unclear, the directional paracrine signalling axis of epithelial FGF9 to stromal FGFR3 has been assumed to play an essential role in prostate homeostasis and the progression of malignancy, because FGF9 expression is relatively low in normal prostate and benign tumours, but significantly elevated in malignant tumours [36].

Since androgen action in the mesenchyme is sufficient for prostate initiation and development [32], paracrine FGF7 and FGF10 in prostate mesenchyme have been proposed to function as androgens to explain the mechanism by which mesenchymal cells regulate epithelial cell development [5,41,42]. However, whether androgens directly regulate FGF7 and FGF10 expression remains controversial. In vitro prostate stromal cell cultures show that both FGF7 and FGF10 mRNA are up-regulated by treatment with testosterone [5,42]; however, observations in vivo indicate that FGF7 is decreased, rather than increased, in response to androgens [43] and the FGF10 mRNA level is not correlated with androgen exposure in prostate organ culture [6]. Whether there are AREs in the promoter regions of Fg7 and Fg10 genes remains to be identified. Moreover, ARs in the mesenchyme may act via indirect pathways to control the expression level of FGF7 and FGF10. Although the importance of androgen signalling in prostate is well known, it remains unresolved whether androgens regulate prostate growth, tissue homeostasis and tissue functions via similar signalling mechanisms. In Fgfr2 conditional null (Fgfr2<sup>cn</sup>) mice [8], the maintenance of dorsolateral prostates is not strictly dependent on androgen. Morphology and histology show that no significant atrophy occurs in adult Fgfr2<sup>cn</sup> mouse prostates within 2 weeks after castration. Similarly, androgen replenishment for castrated Fgfr2<sup>cn</sup> male mice fails to induce dramatic cell proliferation in prostate epithelium. These results demonstrate that FGFR2 signalling is essential for strict androgen dependency in adult prostates with respect to tissue homeostasis. Interestingly, as in control glands, the production of secretory proteins in Fgfr2<sup>cn</sup> prostates is dramatically reduced by androgen deprivation, suggesting that androgen regulation of the secretory function persists in these mutant prostates. Furthermore, neonatal castration of Fgfr2<sup>cn</sup> mice inhibits prostate growth as in control mice. Together, these data suggest that androgens might elicit functions via various pathways in the prostate to regulate gland growth, homeostasis and secretory function.

As a general adaptor for activated FGFR kinases and pathways, FRS2α plays a critical role in mediating functions of the FGF/FGFR signalling axis [26,44]. FRS2α is uniformly expressed in the epithelial cells of developing prostate, but only in basal cells of the mature prostate epithelium. Recent studies [12] showed that ablation of Frs2α in prostatic epithelial precursor cells impairs prostatic ductal branching morphogenesis and compromises cell proliferation (Figure 4C). Importantly, this ablation also inhibits the initiation and progression of prostatic autochthonous tumorigenesis [12]. MAPK and Akt/PI3K are the two main intracellular kinases which are activated by FGFRs through the phosphorylated FRS2α adaptor protein [26]. Prostate organ culture has shown that inhibition of ERK

**Figure 3 Androgen and FGF signalling in the prostate**

Androgen and reciprocal FGF signalling in prostate stromal and epithelial cell interaction. E, epithelial cells; F7, F9 and F10, FGF7, FGF9 and FGF10 respectively; N, nucleus; N.E., neuroendocrine epithelial cells; R2b, FGFR2IIIb; R3c, FGFR3IIIc.
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Reciprocal interactions between stromal and epithelial compartments are critical for prostate development and homeostasis. Alterations in this homeostatic balance can lead to neoplastic transformation when epithelial cells escape the restraints imposed by the local environment. Many components of the FGF signalling system are often found aberrantly expressed in prostate tumours [45,46]. Despite the high homology between the amino acid sequences of FGFR1 and FGFR2, especially within the kinase domain, accumulating evidence indicates that signals elicited by these two FGFRs are different in many tissues, including the prostate [27,47]. The FGFR2IIb isoform is frequently lost or alternatively spliced to FGFR2IIc in epithelial cells during progression to malignancy [48–50], and overexpression of FGFR2IIb in neoplastic cells can restore cell differentiation [47,51]. In contrast, fully malignant prostate tumours often ectopically express the FGFR1 kinase that is normally restricted to the stromal compartment. This ectopic expression promotes autonomous growth of tumour cells and establishes an autocrine loop with abnormally expressed autocrine FGFs [27,37,46,47]. Furthermore, the combination of ectopic FGFR1 with reduction in FGFR2 induces high-grade PIN (prostatic intraepithelial neoplasia) in mice [52]. Such evidence indicates that ectopic FGFR1 and endogenous FGFR2 in epithelial cells have opposite effects on intercompartmental homeostasis in the prostate. Additionally, enhanced expression of FGF10 in prostate stroma or FGFR1 in prostate epithelium induces prostate malignancy [10,11]. These data strongly demonstrate the critical roles of paracrine FGF signalling in mediating the stromal–epithelial interactions which regulate prostate homeostasis and tumorigenesis. Besides the aberrant expression of FGF ligands and FGFRs, FRS2α, a docking protein in the FGF signalling axis that is expressed only in stromal and basal cells of mature prostate epithelium, is apparently expressed in the luminal epithelial cells of regenerating prostates and prostate tumours [12]. The coincidence of the ectopic appearance of FGFR1 coupled with FR2α in malignant prostate epithelial cells, together with evidence that ablation of FR2α inhibits prostate tumorigenesis in the TRAMP (transgenic adenocarcinoma mouse prostate) mouse model, demonstrates that the FGFR1/FRS2α signalling axis is an essential feature of prostate tumorigenesis.

In summary, the spatial and temporal expression and activation of the FGF signalling axis is essential for prostate development, homeostasis and normal secretory function. These processes exemplify the importance of paracrine cross-talk mediated through a diverse set of ligands and receptors, which are compartmentalized in the prostate stroma or epithelium. Finally, aberrant expression of the FGF signalling axis in either compartment of the prostate can adversely affect this homeostatic balance and lead to prostate carcinogenesis.
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