Involvement of Fibroblast Growth Factors and Their Receptors in Epididymo-Testicular Descent and Maldescent

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Abstract

Maldescent of the epididymo-testicular unit can occur as an isolated event or as a component of various syndromes. When part of a syndrome, crypto-epididymis is usually accompanied by other genital and/or extragenital features. Epididymis development is primarily regulated by androgens, and successful epididymo-testicular unit development and descent requires an intact hypothalamic-pituitary-gonadal axis. The developing gonadotropin-releasing hormone system is essential for epididymo-testicular descent and is highly sensitive to reduced fibroblast growth factor (FGF) signaling. Our understanding of the impact of FGFR1 in the process of epididymo-testicular descent has recently improved. At later stages of embryonic development, the undifferentiated epididymal mesenchyme is a specific domain for FGFR1 expression. The majority of individuals with syndromic crypto-epididymis, as well as individuals with isolated maldescent of the epididymo-testicular unit, exhibit some disturbance of FGF, FGFR1 and/or genes involved in hypothalamic-pituitary-gonadal axis regulation. However, the mechanisms underlying FGF dysregulation may differ between various syndromes. © 2016 The Author(s) Published by S. Karger AG, Basel

Key Words

Androgens · Epididymo-testicular descent · FGF8 · FGFR1 · GnRH · Gubernaculum epididymis
testis towards the developing scrotum during the process of inguinal-scrotal descent (fig. 1). The epididymis precedes the testis throughout the entire process of descent into the scrotum. Disturbances in epididymis development can result in a cryptorchid position of the unit [Rachmani et al., 2012; Hadziselimovic, 2015]. Maldescent can occur as an isolated event, or as part of a variety of syndromes. Crypto-epididymis as a syndrome component is usually accompanied by other genital and/or extragenital features that most often result from single-gene abnormalities.

Gonadotropin-releasing hormone (GnRH) neurons are essential for epididymo-testicular descent and onset as well as reproduction maintenance. Proper specification of GnRH cell fate, both prenatally and postnaturally, relies on fibroblast growth factors (FGFs) [Rochester et al., 2012], and the developing GnRH system is highly sensitive to reduced levels of FGF signaling [Chung et al., 2008]. FGFs comprise a family of homologous polypeptide ligands that bind to their cognate fibroblast growth factor receptors (FGFRs). Various FGFs are classified as intracrine, paracrine or endocrine factors [Itoh et al., 2015]. Paracrine and endocrine FGFs are secreted signaling molecules that act via cell-surface FGFRs [Itoh et al., 2015]. FGF-mediated signaling is involved in mitogenesis, proliferation, differentiation, cellular migration, angiogenesis, and tissue injury repair.

Humans possess 22 FGF ligand genes comprising 6 subfamilies that differ in phylogeny and sequence homology. The ligand specificities of FGFR1, FGFR2 and FGFR3 are partially determined by alternative splicing within the C-terminal half of the third immunoglobulin loop of the extracellular FGF-binding domain [Kelleher et al., 2013]. Such alternative splicing creates a IIib isoform (containing exon 8) that is preferentially expressed in epithelial cells and a IIic isoform (containing exon 9) that is preferentially expressed in mesenchymal cells. The IIib isoform preferentially binds FGF ligands that are secreted from adjacent mesenchyme, while the IIic isoform usually binds ligands secreted from the adjacent epithelium [Kelleher et al., 2013]. The configuration of this paracrine arrangement has the benefit of obviating inadvertent autocrine stimulation [Kelleher et al., 2013].

Role of Gonadotropin-Releasing Hormone and FGFs

FGFR1 hypomorphy severely reduces the total number of GnRH neurons, thus inducing congenital hypogonadotropic hypogonadism (CHH), either alone or in association with other hypothalamic-pituitary deficiencies [Chung et al., 2008]. The largest study of CHH diagnosed during childhood found genetic causes for ~30% of CHH cases, and reported that 39% of the cases were associated with malformations and syndromes, most frequently Kallmann syndrome and CHARGE syndrome [Vizeneux et al., 2013]. Both Kallmann syndrome and crypto-epididymis can reportedly be caused by FGFR1 mutation [Pitteloud et al., 2006].

Supporting the importance of GnRH in this developmental process, severe hypoplastic crypto-epididymis has been observed in transgenic mice with migratory arrest of GnRH neurons [Radovick et al., 1991], in hypogonadal mice lacking GnRH [Charlton et al., 1983], and

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**Fig. 1.** Sagittal sections of epididymo-testicular descent in humans of 43–350 mm crown–rump length [Hadziselimovic, 1983]. The gubernaculum (G) inserts proximally into the Wolffian duct (WD, double arrows) and cauda epididymis (CE). The epididymis (E) precedes the testis (T) throughout the entire process of descent into the scrotum. The processus vaginalis (Pv), external oblique fascia (EO), kidney (K), mesonephros (GM), head of the epididymis (Cp), and tail of the epididymis (CE) are indicated. The figure is reproduced from Hadziselimovic [1983] with permission.

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DOI: 10.1159/000444033

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in mice with loss-of-function mutations in the GnRH receptor gene [Pask et al., 2005]. Hypogonadal male mice lacking GnRH are cryptorchid but have a normal gubernaculum, and gonadotropin treatment leads to normal testes development and descent [Charlton et al., 1983]. In cryptorchid boys, GnRH treatment reportedly induces increased testosterone secretion and stimulates further epididymis development and completion of epididymo-testicular descent [Bica and Hadziselimovic, 1993]. Boys with successful descent of the epididymis and testis had a normal-sized epididymis, while the majority of nonresponders to hormonal treatment had a small and irregular epididymis [Bica and Hadziselimovic, 1993].

Luteinizing hormone (LH) also seems to play an important role, as LH receptor knockout mice exhibit bilateral cryptorchidism that can be corrected by testosterone replacement therapy. Specifically, this therapy reverses all of the morphological and gene expression changes in the knockout mice, except those related to insulin-like factor 3 (Insl3), suggesting that testosterone rather than INSL3 facilitates completion of testicular descent [Yuan et al., 2006]. Furthermore, in 66% of naturally cryptorchid mice, treatment with LH-releasing hormone reportedly induces epididymo-testicular descent, while increasing testosterone secretion and normalizing morphology of the underdeveloped cryptorchid epididymis [Hadziselimovic, 1981]. Notably, FGFR1 gene mutations have been described in cases of idiopathic hypogonadotropic hypogonadism and crypto-epididymis [Dodé et al., 2003; Pitteloud et al., 2006].

In 2010, we reported impaired FGFR1 expression in the undescended testis of unilateral cryptorchid boys [Hadziselimovic et al., 2010]. Additionally, decreased FGFR1 protein levels have been found in cryptorchid epididymides of both humans and rodents (fig. 2, 3). These findings support the involvement of FGFR1 in regulating epididymal mesenchyme development. It appears likely that the impaired FGFR1 protein secretion found in underdeveloped mesenchyme in cryptorchid humans and

**Androgens and FGFR1**

Androgens are the primary factors regulating epididymal development and function. However, a large body of evidence now suggests that growth factors also play important roles in epididymis regulation and maintenance. Over recent years, it has become clear that FGF signaling is involved in the development and normal functioning of male reproductive organs, such as the testis and epididymis [Cotton et al., 2008]. For example, Fgf10, is expressed in the mesenchyme of the lower Wolffian ducts and regulates epithelial growth of the seminal vesicles and prostate [Archambeault et al., 2009]. It has also been reported that testosterone treatment increases Fgf10 transcription in the seminal vesicles [Thomson and Cunha, 1999]. At later stages of epididymal development, FGFR1 is specifically expressed in the undifferentiated mesenchyme [Basilico and Moscatelli, 1992; Cotton et al., 2008]. Moreover, FGFR1 gene mutations have been described in cases of idiopathic hypogonadotropic hypogonadism and crypto-epididymis [Dodé et al., 2003; Pitteloud et al., 2006].

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rodents contributes to the defective epididymis forma-
tion and the consequent undescended position (fig. 2, 3).

Müllerian-Inhibiting Substance and INSL3

Nef and Parada [1999] reported normal epididymis
development in Insl3-deficient cryptorchid mice, with no
impairment of descent. In contrast, we found that Insl3
mutant mice showed absent smooth musculature around
the epididymal duct, which resulted in a high intra-
abdominal undescended position [Hadziselimovic and
Adham, 2007]. This finding supports the importance of
an intact epididymis for descent of the epididymo-testicu-
lar unit. Emmen et al. [2000] reported that Insl3 is not
essential for Wolffian duct growth, and that the Mülleri-
an-inhibiting substance (MIS) does not influence guber-
naculum growth. Moreover, mice with mutations of Mis
and the MIS receptor, and mice with intrauterine immu-
nization against MIS show normal epididymo-testicular
descent and normal scrotum development [Picar et al.,
1983; Behringer et al., 1994; Bartlett et al., 2002]. Al-
though MIS has no obvious effect on testicular descent in
mice, it is still considered to potentially affect human de-
scent [Hutson et al., 2015]. Those authors investigated
boys with androgen insensitivity and found testicle local-
ization in the inguinal region, indicating that the ‘first
phase’ of descent is androgen independent and MIS de-
pendent [Clarnette et al., 1997]. In contrast, abdominal
testes were reported in 86% of patients showing a com-
plete female phenotype, with a decreasing incidence cor-
responding with increasing masculinization [Barthold et
al., 2000]. Thus, in patients with complete androgen in-
sensitivity, testicular position correlates with the genital
phenotype, supporting a major role for androgens in
epididymo-testicular descent.

Syndromes Associated with Crypto-Epididymis

A large number of syndromes are associated with mal-
descent of the epididymo-testicular unit (table 1). Hypo-
gonadism is also established in many of these conditions,
often based on central or hypothalamic-pituitary deficit
[Pinsky et al., 1999]. In majority of studied syndromes,
affected individuals with crypto-epididymis also exhibit
various forms of mental retardation (table 1). The EGR4,
FMR2 (AFF2), and VCX3A genes encode proteins in-
volved in signaling pathways that contribute to intel-
ctual and cognitive functions. Thus, the impaired expres-
sions of these genes in cryptorchid boys with high infertil-
ity risk could explain the increased odds ratio for the low
IQ observed among boys with undescended testes
[Hadziselimovic et al., 2014].

Kallmann syndrome is a classic example of a congeni-
tal syndrome associated with hypothalamus-pituitary-
gonadal axis impairment. Clinical findings also indicate
that some developmental defects observed in CHAR
e syndrome may be caused by insufficient FGF signaling
levels. For example, CHARGE syndrome shows substan-
tial clinical overlap with 22q11.2 deletion and Kallmann syndrome, which are both linked to reduced FGF signaling [Randall et al., 2009; Scambler, 2010; Corsten-Janssen et al., 2013; Miraoui et al., 2013]. Mutations in the same exon of \(FGFR2\) are observed in Crouzon, Jackson-Weiss and Pfeiffer syndrome [Meyers et al., 1996]. Mutations elsewhere in the same gene also present with a common phenotype – for example, in Crouzon syndrome with

<table>
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<tr>
<th>Frequent</th>
<th>Occasional</th>
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<tr>
<td>Aarskog-Scott (faciogenital dysplasia)*</td>
<td>Ablepharon-Macrostomia</td>
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<td>Apert*</td>
<td>Anophthalmia-Esophageal-Genital</td>
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<td>C syndrome (Opitz trigonocephaly)*</td>
<td>Ataxia-Telangiectasia*</td>
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<td>Carpenter*</td>
<td>Beckwith-Wiedemann*</td>
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<td>Cockayne*</td>
<td>Bird-Headed Dwarfism (Seckel and others)*</td>
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<td>Dubowitz*</td>
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<td>Chudley Mental Retardation*</td>
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<td>Fetal-face syndrome (Robinow)*</td>
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<td>FG syndrome (Opitz-Kaveggia)*</td>
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<td>Oculocerebral (Cross)*</td>
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<td>X-linked mental retardation, gynecostasia, and hypogonadism*</td>
<td>Opitz G/BBB Compound*</td>
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* Syndromes associated with different degrees of mental retardation, adapted according to Pinsky et al. [1999].
mutations in either exon IIIa or in IIIc [Meyers et al., 1996]. Moreover, disorders such as Apert’s syndrome are associated with incorrect FGF ligand binding and inappropriate FGFR autocrine activation [Kelleher et al., 2013].

The discovery of mutations in FGFR1 and FGF8 in CHH indicates a previously unappreciated role of FGF8-FGFR1 signaling in GnRH neuron ontogeny [Miraoui et al., 2013]. Fgf8 hypomorphomic mice show a lack of GnRH neurons in the hypothalamus, demonstrating the exquisite sensitivity of the GnRH neuronal population to FGF8 signaling, which appears to be androgen dependent [Gnanapragasam et al., 2002]. More recent studies establish FGF8 as a critical morphogen for GnRH neuron fate specification and for olfactory system development [Miraoui et al., 2013]. Using expression data from diverse organisms, Miraoui et al. [2013] identified a cluster of genes that are similarly expressed and regulated during development, which are collectively referred to as the ‘FGF8 synexpression’ group. These genes show spatiotemporal expression patterns similar to that of FGF8, as well as act as enhancers or inhibitors, specifically modulating the signaling efficiency of FGF8 through FGFR1. Investigating FGFR1 mutations in CHH has provided pivotal data, challenging the traditional monogenic view of this disorder and guiding the transition towards more complex genetic models [Pitteloud et al., 2007].

Five additional gene mutations in FGFR1, IL17RD, DUSP6, SPRY4, and FLRT3 have now been observed in CHH [Miraoui et al., 2013]. Moreover, the mechanisms underlying FGF dysregulation may differ among different syndromes.

**Conclusion**

Many cases of syndromic crypto-epididymis, as well as a majority of isolated cases, have either a disturbance of FGFs, FGFR1, FGFR3, and/or a disturbance of the genes involved in regulating the hypothalamic-pituitary-gonadal axis in common [Hadziselimovic et al., 2009; Hadziselimovic et al., 2010]. Normally, muscle development requires signaling by members of the FGF family and their downstream effector EGR1 [Nentwich et al., 2009]. Deficient expression of EGR4 and EGR1 was observed in cryptorchid boys [Hadziselimovic et al., 2009]. Thus, the observed decrease in FGF expression may result in decreased EGR gene expression, inducing CHH and impaired epididymal mesoderm development, resulting in maldescent of the epididymal-testicular unit.

**Statement of Ethics**

The Institutional Review Board and the Independent Ethics Committee of the Children’s Clinic Liestal approved all aspects of this study, which were in accordance with the Declaration of Helsinki. Approval was provided for research involving the use of material (data records or biopsy specimens) that had been collected for non-research purposes.

**Disclosure Statement**

The authors declare no conflicts of interest.

**References**


DOI: 10.1159/000444033

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