Stem Cell Research 24 (2017) 174–180

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

# Regulation of fetal male germ cell development by members of the TGFβ superfamily

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ARTICLE INFO

*Article history:*
Received 13 December 2016
Received in revised form 4 May 2017
Accepted 15 July 2017
Available online 19 July 2017

*Keywords:*
Germ cells
TGFβ
Activins
Nodal
Cripto

ABSTRACT

There is now substantial evidence that members of the transforming growth factor-β (TGFβ family) regulate germ cell development in the mouse fetal testis. Correct development of germ cells during fetal life is critical for establishment of effective spermatogenesis and for avoiding the formation of testicular germ cell cancer in later life. Here we consider the evidence for involvement of various TGFβ family members, attempt to reconcile discrepancies and clarify what we believe to be the likely *in vivo* roles of these factors.



## 1. Introduction

During mammalian fetal development, germ cells migrate to the nascent gonads, colonise them and then respond to signals from somatic cells that make them commit to oogenesis in the fetal ovary or spermatogenesis in the fetal testis (McLaren, 2001) (Fig. 1). In the ovarian environment, retinoic acid (RA) acts directly on germ cells to trigger expression of the critical pre-meiotic gene *Stra8* and thereby drive them to embark on meiosis (Bowles et al., 2006; Koubova et al., 2006; MacLean et al., 2007; Bowles et al., 2010, 2016). It is likely that another signaling pathway is also activated in ovarian fetal germ cells because oogenesis (but not meiosis) can proceed in the absence of *Stra8* (Dokshin et al., 2013). In the testis, endogenous RA is degraded by the P450 enzyme CYP26B1 and, therefore, germ cells do not express *Stra8* and therefore do not initiate meiosis (Bowles et al., 2006; Koubova et al., 2006; MacLean et al., 2007). Instead, they eventually stop proliferating (initiating $G_1/G_0$ mitotic arrest during the 12.5 to 14.5 dpc period) and begin to express characteristic molecular markers that evidence their commitment to the male program of germ cell differentiation, spermatogenesis (Adams & McLaren, 2002; Western et al., 2008; Suzuki & Saga, 2008; La Salle et al., 2004).

One signaling molecule produced by the somatic cells of the developing testis is FGF9. This factor has an important role in development of the somatic tissue of the testis because when it is genetically deleted testicular development is compromised, even to the extent of frank male-to-female sex reversal (Colvin et al., 2001; Schmahl et al., 2004). Besides this essential role in somatic testis development, FGF9 also acts directly on testicular germ cells to maintain expression of pluripotency markers, to make germ cells less prone to succumb to meiosis and, eventually, to support the expression of male fate markers *Nanos2* and *Dnmt3L* (Bowles et al., 2010; Barrios et al., 2010; Tian-Zhong et al., 2016). Testicular germ cells express various FGF receptors (Bowles et al., 2010), however direct downstream targets of FGF signaling in these cell types remain unknown.

Following the initial and as-yet undefined actions of FGF9, there is evidence that TGFβ signals are the major drivers of the commitment to the male germ cell fate, including initiation of cell cycle arrest as well as the expression of key fate markers. The transforming growth factor beta (TGFβ) superfamily is composed of over 40 cytokines in mammals, including TGFβ isoforms-1,-2 and -3, growth differentiation factors (GDFs), Nodal, Activins and Inhibins, which are considered to make up one major branch of the family and, in the other branch, the bone morphogenic proteins (BMPs). This major subdivision into two families is based on the downstream pathways activated (see below) [reviewed by (Miyazawa et al., 2002)]. Members of this superfamily are involved in a range of cellular processes including control of cell proliferation, differentiation, apoptosis, migration and cell fate specification [reviewed by (Kitisin et al., 2007)]. Here

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http://dx.doi.org/10.1016/j.scr.2017.07.016

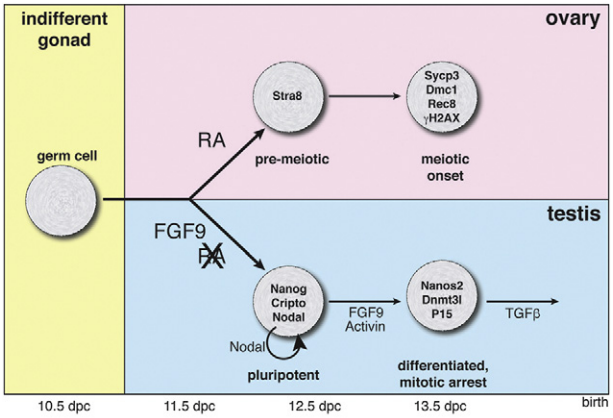


**Fig. 1.** Regulation of germ cell development during fetal life, in the mouse model. Primordial germ cells begin to colonise the sexually-indifferent gonad at about 10.5 dpc. In the developing ovary, retinoic acid (RA) present in the somatic environment acts directly on germ cells to stimulate expression of the critical pre-meiotic factor Stra8. Germ cells subsequently enter meiosis and express Sycp3, Dmc1, Rec8 and γH2AX. In the developing testis endogenous RA is degraded by Cyp26b1, a P450 enzyme expressed by somatic cells (not shown) and, for this reason, testicular germ cells do not express Stra8 and do not enter meiosis. Sertoli cells produce FGF9 which acts directly on germ cells to stimulate expression of Cripto, the obligate co-receptor for Nodal. The presence of Cripto on the germ cell surface allows auto-upregulation of Nodal which feeds back on germ cells to maintain expression of pluripotency markers, particularly Nanog. When FGF9 is present at low concentrations (from 12.5 dpc) it acts directly on germ cells to induce expression of male fate markers Nanos2, Dnmt3l and P15. Activins and/or TGFβs may act directly on germ cells to stimulate male fate marker expression, to induce mitotic arrest and to maintain the quiescent state.

we focus on members of the TGFβ arm and particularly Nodal, Activins and TGFβ, all of which have been implicated in fetal testicular germ cell development.

## 2. TGFβ signaling

TGFβ ligands act as homo- or heterodimers and signal through cell surface serine/threonine kinase receptor complexes composed of two types of receptors [reviewed by (Shi & Massague, 2003)] (Fig. 2). When ligand binds to the extracellular domain of Type II receptors (TGFβR2 for TGFβ, ACVR2A/ACVR2B for Activin and Nodal) the Type II receptor transactivates a Type I receptor (Activin receptor-like kinases (ALKs): ALK5 for TGFβs (also known as TGFβRI) and ALK2, -4 and -7 for Activin and Nodal) by phosphorylation. BMP ligands use a different set of Type I and Type II receptor molecules including ALK2, -3, -6 and BMP receptor type II (BMPR2) in addition to ACVR2A/ACVR2B. Activated receptor complexes in turn phosphorylate intracellular effector proteins of the mothers against decapentaplegic (SMAD) family which subsequently interact with the common mediator SMAD4; the complex then translocates to the nucleus where it acts, together with co-activators and co-repressors, to directly regulate gene transcription. SMAD2 and SMAD3 are the effectors of TGFβ, Activin and Nodal signaling whilst SMAD1, -5 and -8 respond to BMP-activated receptors.

Considerable overlap exists in the receptors used by the various ligands as well as in SMAD proteins employed and, therefore, extensive redundancy is to be expected. Although Nodal and Activin share the same receptors, Nodal requires the additional presence of an obligate co-receptor Cripto (also known as TDGF1, a member of the epidermal growth factor-Cripto-FRL1-Cryptic (EGF-CFC) family) to successfully signal through the complex (Shen & Schier, 2000). Cripto is a small GPI-anchored protein that binds, through its CFC domain, to Type I receptor, ALK4, and through its EGF-like domain to Nodal, the overall effect being to greatly potentiate Nodal signaling. Although the presence of Cripto is crucial for Nodal signaling there is evidence that it attenuates Activin signaling: it is reported that when Cripto forms a complex with Type I and Type II receptors it diminishes the strength of Activin signaling by about 50% (Kelber et al., 2008; Gray & Vale, 2012). Hence in the absence of Cripto, Nodal cannot signal at all but Activin signals very strongly and, therefore, genes responsive to high levels of SMAD2/3

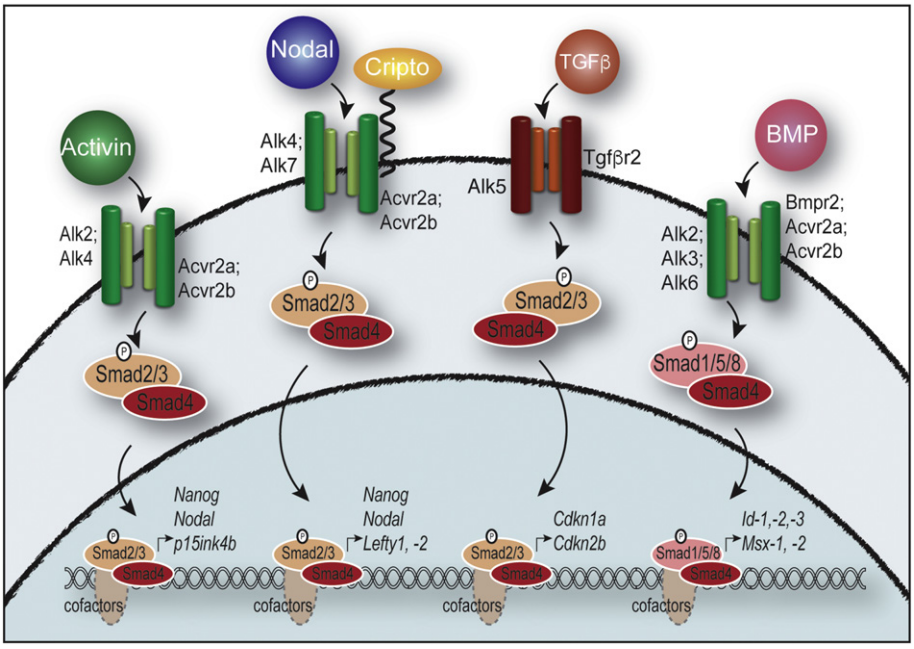


**Fig. 2.** TGFβ signaling pathway components. Each TGFβ morphogen (Activin, Nodal, TGFβ and BMP) signal through different combinations of Type I and Type II receptor kinases. Type I receptors include Alk2, -3, -4, -5, -6 and -7. Type II receptors include Acvr2a, Acvr2b, Tgfβr2 and Bmpr2. Activated receptor complexes phosphorylate specific intracellular SMAD effector proteins (SMAD1, -2, -3, -5 and -8) which subsequently interact with the common mediator SMAD4. The SMAD complex translocates to the nucleus where it acts, together with co-activators and co-repressors, to directly regulate gene transcription. Examples of several gene targets for each pathway are depicted, however this list is by no means exhaustive and is cell-context specific.