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Editorial Commentary

SMAD Expression in the Testis Predicts Age- and Cell-Specific Responses to Activin and TGFß

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Xu J, Beyer AR, Walker WH, McGee SA. Developmental and Stage-Specific Expression of Smad2 and Smad3 in rat testis. *J Androl.*2003 ;24:192–200.[Abstract/Free Full Text]

Ligands in the transforming growth factor ß (TGF ß) superfamily play diverse roles throughout the body in both developing and mature animals. In mammalian reproductive systems, ligands from the subfamilies of activin, inhibin, TGFß, growth differentiation factor (GDF), and bone morphogenetic protein (BMP) have all been shown to have effects at some, if not all, levels of the hypothalamo-pituitary-gonadal axis. The activins and inhibins were, in fact, identified on the basis of their ability to stimulate and suppress pituitary follicle-stimulating hormone (FSH) synthesis and secretion. In adult animals, inhibins are produced by testicular Sertoli cells and granulosa cells of developing ovarian follicles (as well as by luteal cells in primates). These proteins act in an endocrine fashion to suppress FSH from the pituitary. Although it had been believed that activins stimulated FSH through an endocrine mechanism, it is now clear that most circulating activins are of nongonadal origin and are biologically inactive because they are bound to bioneutralizing follistatins (reviewed in Risbridger and Cancilla, 2000). It now appears that activins synthesized in the pituitary (activin B in particular) stimulate FSH through a paracrine or autocrine mechanism.

In addition to their pituitary actions, activins (and other TGFß superfamily members) have potent autocrine/paracrine actions within the gonads. For example, the adult testis produces the activin/inhibin α and ßB subunit proteins, and therefore can produce both activin B and inhibin B. There is some controversy regarding the production the inhibin/activin ßA subunit in the adult testis, but circulating inhibin A is not detectable in males suggesting that the ßA subunit is not produced at significant levels (or at least not in cells that also synthesize the inhibin α subunit). Activins have several effects within the testis. For example, exogenous activin A inhibits LH- or hCGstimulated testosterone production by Leydig cells (Hsueh et al, 1987; Lin et al, 1989). The TGFß ligands, which use similar intracellular signaling proteins as the activins (see below), produce similar effects (Lin et al, 1987; Gautier et al, 1997). It is unclear whether or not inhibins directly affect LH-stimulated testosterone production by Leydig cells, but they do appear to antagonize activin's effects (Hsueh et al, 1987; Lin et al, 1989).

It is interesting that activins can stimulate and inhibit cell proliferation in different testicular cell types, but these effects appear to be age-dependent. For example, in testis fragments from 9-day old rats, activin A in combination with FSH stimulated Sertoli cell proliferation. The same treatment was without effect in testicular fragments from rats at 18 days of age (Boitani et al, 1995). There is some suggestion that age-related changes in activin receptor expression may mediate this change in sensitivity to activin (Fragale et al, 2001), but the results reported by Xu et al in this issue of the *Journal of Andrology* may shed some additional light on this and other age-related changes in activin action in the rat testis.

Like other members of the TGFß superfamily, activins affect target cells by binding to a receptor serine/threonine kinase receptor (RSK) complex. The complex consists of a ligand binding subunit, the type II receptor, which recruits and phosphorylates a second RSK (the type I receptor), which propagates intracellular signals. Activins bind to one of two type II receptors, ActRIIA or ActRIIB. As many as five variants of the latter receptor have been described, although one form, ActRIIB2, appears to be most abundant. After activin binds the type II receptor, the type I receptor, ALK4, is recruited into the complex and is phosphorylated by the constitutively active kinase domain of the type II receptor. This phosphorylation event activates the catalytic subunit of ALK4, which, like the type II receptor, has serine/threonine kinase activity. Although all the downstream targets of ALK4 have not been identified (Attisano and Wrana, 2002), it is clear that much of activin signaling is dependent on activation of proteins in the SMAD family, specifically SMAD2 and SMAD3. Both SMAD2 and SMAD3 are rapidly phosphorylated on conserved C-terminal serine residues by the activated ALK4. This phosphorylation causes the SMADs to dissociate from the receptor complex and bind to a cofactor protein, SMAD4, in the cytoplasm. The activated SMAD complex then translocates to the nucleus, where it affects target gene transcription in concert with coactivator and corepressor proteins (Attisano and Wrana, 2002). The TGFß ligands use different type I and type II receptors than the activins, but they also stimulate SMAD2 and SMAD3 phosphorylation. Other TGFß superfamily members, like the BMPs, signal through different receptor-regulated SMADs (SMADs 1, 5, and 8).

Several studies have described the expression of activin type II receptors in adult and developing testes (Woodruff et al, 1992; Feng et al, 1993; Cameron et al, 1994). At least one report has also indicated the expression of ALK4 in spermatids (De Jong, 1997). Surprisingly few papers have described SMAD expression in mammalian testes (Wang and Zhao, 1999; Goddard et al, 2000; Kano et al, 2001; Luukko et al, 2001). In this issue, Xu et al make an important contribution in this regard by describing postnatal expression profiles for both SMAD2 and SMAD3 in the rat testis. Their results show that both the abundance and cellular localization of these SMADs change from postnatal Day 10 to adulthood. Specifically, testicular SMAD2 protein levels are higher during juvenile development than in adulthood. SMAD3 levels show a similar yet less pronounced developmental profile. Protein localization studies further characterized developmental changes in SMAD expression. For example, both SMAD2 and SMAD3 were detected in Sertoli and Leydig cells of 10-day old animals. Whereas both SMADs continued to be expressed in adult Sertoli cells, they were virtually absent in adult Leydig cells. Surprisingly, neither SMAD was detected in spermatogonia at any age. Activin receptors are expressed in spermatogonia and activins affect spermatogonial proliferation (Mather et al, 1990; Woodruff et al, 1992; Kaipia et al, 1993; Boitani et al, 1995). These results raise the interesting possibility that activins may signal in a SMAD-independent manner in this cell type.

Although SMAD2 and SMAD3 appear to play redundant functions in various biological systems, it is clear that the two proteins also mediate distinct cellular responses. For example, the phenotypes of SMAD2- and SMAD3-deficient mice differ dramatically (Weinstein et al, 2000). The two SMADs have different DNA-binding properties, and this accounts for some of the differences in their mechanisms

and modes of action (Labbe et al, 1998; Yagi et al, 1999). However, it is possible that temporal or spatial differences in their expression may also contribute. In the testis, Xu et al show that SMAD2 and SMAD3 are often but not always expressed in the same cell types or cellular compartments at the same times. Thus, variability in patterns of SMAD expression may contribute to differences in responsiveness of testicular cell types to activins at various stages of life.

The data presented by Xu et al will no doubt provide a framework for future, hypothesis-driven research. For example, the pronounced age-related decline in Leydig cell SMAD2 and SMAD3 levels predicts that the inhibitory effects of activins (and TGFßs) on LH-stimulated testosterone production will be less pronounced (if not completely absent) in Leydig cells derived from adult animals than from younger animals. Moreover, the overall, age-related reduction in SMAD2 and SMAD3 protein levels in rat testis predicts that activins (and TGFßs) may play more important roles in testicular development than in adult testicular function.

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