IJAE
Vol. 118, n. 1 (Suppl.): 32-33, 2013
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Mechanisms of INSL3 signaling in male reproductive organs

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Summary

Global ablation of INSL3 hormone or its receptor RXFP2 in male mice results in cryptorchidism and infertility. Using novel LacZ knock-in *Rxfp2* allele we demonstrated a strong expression of this gene in postmeiotic germ cells. RXFP2 was expressed in embryonic and neonatal gubernaculum. No RXFP2 expression was detected in cremaster muscles in adult mice. We produced a floxed allele of *Rxfp2* and then deleted this gene in male germ cells in testes located in normal scrotal position. No differences in fertility or spermatogenesis of such males were found, suggesting non-essential role of INSL3 signaling in germ cell differentiation in adult males. We have also produced shRNA transgenic mice with reduced RXFP2 expression Such males manifested various degree of uni- and bilateral cryptorchidism. Total gene expression analysis of the mutant cremasteric sacs indicated misexpression of a significant number of genes in Wnt/β-catenin and NOTCH pathways. Conditional deletion of β-catenin or *Notch1* genes in male gubernacular ligament resulted in its abnormal development. Our data suggest that β-catenin and NOTCH1 pathways are potential targets of INSL3 signaling during gubernacular development.

Key words

INSL3, RXFP2, testis, cryptorchidism, spermatogenesis.

The deletion of Insulin-like 3 peptide (INSL3), a major secretory product of Leydig cells, or its G protein-coupled receptor RXFP2 in mouse males causes high intra-abdominal cryptorchidism associated with complete infertility (Agoulnik, 2005). Different methods used to establish the expression profile of RXFP2 led to the contradicting results. It was unclear whether INSL3 signaling ablation itself had any effect on spermatogenesis in testis located in normal scrotal position. Due to a complete arrest of gubernacular development in mutants it is difficult to determine the downstream cellular pathways affected by INSL3/RXFP2 deletion. To address these questions we produced a series of new mutants. First, the knock-in RXFP2 allele with IRES-LacZ reporter cassette inserted into one of the gene introns was produced (Huang et al., 2012). We also generated a conditional floxed RXFP2 allele and the shRNA transgene which expressed shRNA targeting RXFP2 (Huang *et al*., 2012, Kaftanovskaya *et al*., 2011). Strong RXFP2 expression was detected in postmeiotic germ cells, mesenchymal and epithelial cells of embryonic gubernaculum, in non-differentiated fibroblasts within adult cremaster muscles. Using Cre transgene driven by retinoic acid receptor beta promoter, conditional gene targeting in gubernacular cells at early embryonic

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stages caused high intraabdominal cryptorchidism as in males with a global deletion of RXFP2. When the RXFP2 was deleted in gubernacular smooth or striated muscle cells, no abnormalities of testicular descent were found. Specific ablation of RXFP2 in male germ cells did not affect testis descent, spermatogenesis, fertility, or germ cell apoptosis in adult males. We have shown that the shRNA transgene reduced RXFP2 expression, leading to the variable degrees of uni- and bilateral cryptorchidism. Gene expression analysis of a mutant cremasteric sac using RNA microarrays indicated abnormal expression of a significant number of genes in Wnt/β-catenin and NOTCH pathways. Males with the deletion of β-catenin or *Notch1* in the gubernacular ligament demonstrated abnormal development. Our data indicate that β-catenin and NOTCH pathways are potential targets of INSL3 signaling during gubernacular development and that the INSL3/RXFP2 signaling is dispensable for spermatogenesis and fertility in adult males.

Acknowledgments: This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Institute of Health R01HD37067 grant to A.I.A.

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