

Expression and localization of RLF/INSL3 receptor RXFP2 in boar testes

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Summary

This study investigated the possibility of the presence of specific receptor for relaxin-like factor (RLF)/insulin-like peptide 3 (INSL3) in boar testes. While RLF/INSL3 was produced by Leydig cells in the boar testis, its own receptor RXFP2 was expressed mainly in meiotic and post-meiotic germ cells, but not in Leydig cells, suggesting the existence of RLF/INSL3–RXFP2 signaling in germ cells of boars.

Key words

RLF/INSL3; RXFP2; expression; localization; testis; male pig

Introduction

Relaxin-like factor (RLF), also known as insulin-like peptide 3 (INSL3), is a new member of the relaxin/insulin family that is expressed in testicular Leydig cells. While the understanding of the function of RLF/INSL3 is accumulating in rodents (Kawamura *et al.*, 2004; Anand-Ivell *et al.*, 2006), its function in adult boars is unknown. Recently, we have isolated native RLF/INSL3 from boar testes and demonstrated that native RLF/INSL3 is a 12 kDa monomer comprising three domains B–C–A with biological activity (Minagawa *et al.*, 2012). As the first step towards elucidating the potential function of the RLF/INSL3 in boar testes, this study was performed to examine the possibility of the presence of RLF/INSL3 receptor RXFP2 in the boar testis.

Materials and Methods

Testes were collected from Duroc boars. The validation of commercially available antibodies directed against human RXFP2 was performed by the HEK293 cells transiently transfected with Flag-tagged mouse *Rxfp2* construct (Minagawa *et al.*, 2012)

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and then employing double immunofluorescence. RXFP2-expressing cells in boar testis were identified using immunohistochemistry and laser micro dissection followed by RT-PCR. In addition, the dynamics of RXFP2 protein were evaluated by western blotting.

Results and Discussion

Specificity of RLFP2 antibody was validated by indicating the recognition of the receptor molecule in the HEK-293 cells expressing FLAG-tagged RXFP2. Using the antibody, RXFP2 was found to be localized mainly in meiotic and post-meiotic germ cells, but not in Leydig cells (Fig. 1). *RXFP2* mRNA was also detected in the same cell types by which RXFP2 protein was expressed. Furthermore, RXFP2 expression seemed to increase from the pubertal stage onwards. From these findings, an obvious implication is that RLF/INSL3 may act as a paracrine factor in seminiferous germ cells in the testis. Interestingly, intratesticular administration of RLF/INSL3 has been reported to result in substantial decrease of male germ cell apoptosis induced by gonadotropin withdrawal in male rats (Kawamura *et al.*, 2004).

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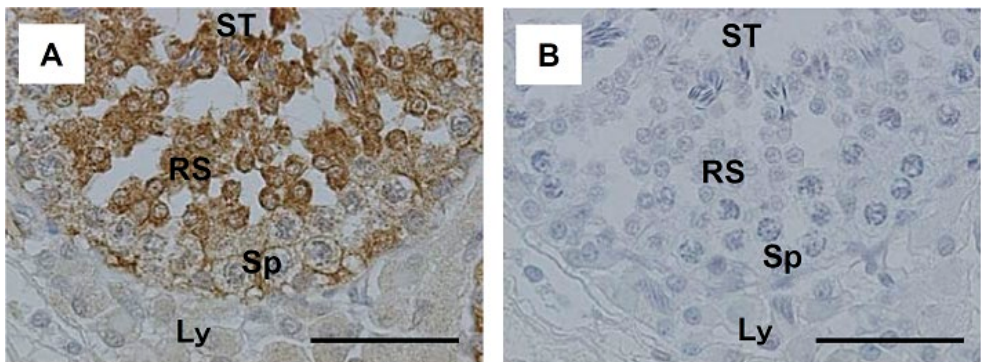


Figure 1. Immunolocalization of RXFP2 protein in the boar testis harvested from 30 weeks of age. **(A)** RXFP2 antibody. RXFP2 was localized mainly in meiotic and post-meiotic germ cells, but not in Leydig cells. **(B)** Absorbed antibody. Signals were effectively blocked. RS, round spermatids; Sp, spermatocytes; ST, seminiferous tubules; Ly, Leydig cells. Bars = 50 μ m.

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