

PROTEIN EXPRESSION PROFILE OF PROTEASE NEXIN-1 (PN-1) DURING THE MURINE TESTIS DEVELOPMENT

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Introduction: Protease nexin-1 (PN-1) is a potent serine protease inhibitor that inactivates several serine proteases, including thrombin, tissue-type and urokinase-type plasminogen activator (PA). It modulates proteolytic activity of PAs, which have been implicated to involve in tissue remodeling, for example, during cycling uterus and follicular rupture at ovulation. In addition, PN-1 gene knockout leads to defeat in male fertility in mice, suggesting that the balance between proteases and their inhibitors is a critical factor in controlling the male fertility. Besides, the role of PN-1 in other male reproductive tissues is still unknown. In this study, we revealed its cellular expression in testis tissues and on sperm.

Methods: The Western blot analysis was used to reveal the specificity and sensitivity of anti-PN-1 antibody. Immunohistochemistry was used to examine the cellular localization of PN-1 in testis tissues and on spermatozoa. Testis from 12.5, 14.5, 16.5, and 18.5 days postcoitus (d.p.c.) and postnatal day 1, 1 week to 8 week old were collected, fixed in formaldehyde and embedded in paraffin to examine PN-1 protein expression during testis development. Sperm from testis and various parts of epididymis were collected and immunostained by PN-1 antiserum.

Results: PN-1 was detected specifically by the anti-PN-1 antiserum. Immunohistochemistry revealed that PN-1 protein was expressed on the spermatogonia, spermatocyte, spermatid, mature sperm and the Leydig cells of adult testis. PN-1 was found in the Sertoli cells at 12.5 to 18.5 d.p.c., postnatal day 1 to 1 week old testis. Positive signal was found on acrosomal vesicle from 4 week onward. Strong signal was found on spermatid and mature sperm. Isolated sperm cell from testis or various parts of epididymis showed PN-1 protein staining on sperm acrosome.

Conclusions: PN-1 may involve in testis tissue remodeling. However, its extensive expression on germ cells may indicate another role during the spermatogenesis. Interestingly, expression of PN-1 on sperm acrosome suggests that it may have effects on sperm physiology. This is the first study to provide a comprehensive protein expression profile of PN-1 during testis development.