P-1 Identification of a Novel Hyaluronidase, Hyal5, Involved in Penetration of Mouse Sperm Through Cumulus Mass

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Background & Objectives: A glycosylphosphatidylinositol (GPI)-anchored hyaluronidase, PH-20, on the sperm surface has long been believed to assist sperm penetration through the cumulus mass surrounding the eggs. However, mouse sperm lacking PH-20 were still capable of penetration the cumulus mass despite a delayed dispersal of cumulus cells. Intriguingly, a 55-kDa hyaluronan-hydrolyzing protein was abundantly present in wild-type and PH-20-deficient mouse sperm. The purpose of this study was to identify of a novel hyaluronidase, and evaluate the effect of this novel hyaluronidase during sperm penetration through the cumulus mass.

Method: PA fraction was prepared from acrosome-reacted sperm (1×10^9 cells), and applied onto a Hitrap heparin HP column that had been equilibrated with 20 mM Tris/HCl (pH 7.5). Proteins were eluted from the column with a linear gradient of 0 to 0.5 M NaCl in the same buffer (15 ml) at a flow rate of 0.5 ml/min. Aliquots of each fraction (0.5 ml) were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) in the presence of hyaluronan. Following two- dimensional PAGE, protein spots on the gels were cut, digested with trypsin, and analyzed by an AXIMA Matrix-Assisted Laser Desorption Ionization/ Time-of-Flight Mass spectrometer according to the manufacturer's protocol. Proteins were identified by the mass fingerprinting method using a MASCOT software.

Results: We have purified the 55-kDa mouse protein from soluble protein extracts released from epididymal sperm by acrosome reaction, and have identified as a novel hyaluronidase, Hyal5. Hyal5 was exclusively expressed in the testis, and formed a 160-kbp gene cluster together with Hyalp1, Hyal4, and ph-20 on mouse chromosome 6. Hyal5 was a single-chain hyaluronidase present on the plasma and acrosomal membranes of sperm presumably as a GPI-anchored protein. Moreover, hyaluronan zymography revealed that Hyal5 is enzymatically active in the pH range 5~7, and inactive at pH 3 and 4. Both Hyal5-enriched, PH-20-free soluble protein extracts, and PH-20-deficient mouse sperm were capable of dispersing cumulus cells from the cumulus mass.

Conclusions: This study describes the identification of a novel hyaluronidase, Hyal5, presumably involved in the sperm penetration through cumulus mass in the mouse. Despite the similarities in the amino acid sequence, chromosomal localization, and gene expression pattern, Hyal5 is distinguished from PH-20 by several respects: the abundance, subcellular localization, and endoproteolytic processing of these two proteins on epididymal sperm.