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Mini Review

Is Sperm Hyaluronidase Indispensable in Mammalian Fertilization?

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Abstract

Fertilization involves several precisely coordinated steps, including sperm dispersal of the oocyte cumulus mass, adhesion and binding to the zona pellucida, penetration through the zona pellucida, and fusion with the oocyte. Hyaluronidase, which is present in the sperm head, is known to be essential for the decomposition of the oocyte cumulus mass. Interestingly, two types of hyaluronidase—SPAM1 and —Hyal5 have been discovered in rodents, while only SPAM1 has been reported in other mammals such as pigs and humans. SPAM1 localizes to the plasma membrane of the sperm, while Hyal5 is present on the plasma and the outer acrosomal membranes. Male mice lacking either one of these two hyaluronidases can fertilize normally, suggesting that neither SPAM1 nor Hyal5 is essential for fertilization in mice. Although it has been 30 years since the discovery of hyaluronidase, its role in fertilization remains unclear. This review focuses on whether hyaluronidase is essential for fertilization in mammals.

The steps in mammalian fertilization include sperm penetration through the cumulus, sperm adhesion and penetration into the zona pellucida (ZP), and fusion of the sperm and oocyte plasma membranes [1-3]. Freshly ovulated mammalian oocytes are surrounded by cumulus cells embedded in an extracellular matrix abundant in hyaluronic acid [2]. Therefore, the first key process in fertilization is the direct interaction between the sperm and the cumulus mass. In fact, *in vitro* studies show that fertilization rate is greatly reduced following removal of the cumulus mass. Hyaluronidases, enzymes present in the sperm head, facilitate sperm penetration by degrading the cumulus matrix. The mammalian genome contains two groups of hyaluronidase genes encoding somatic and testicular forms of the enzyme [4]. These genes are clustered on chromosomes 3p21 and 7q31 in humans and on 3p21 and 6A2 in mice (Figure 1). The Hyal1 was identified as the first somatic hyaluronidase until Hyal2, known as widely tissue expressed acid-active hyaluronidase, had been isolated from the mouse tissue that showed 36.5% identity with the mouse SPAM1 [5]. Meanwhile, The genes in the testicular group, Hyal4, Hyal6, SPAM1 and Hyal5 in mice, exhibit either tissue-specific expression, albeit Hyal6 has no functional protein in human [6]. The most widely studied testis-specific hyaluronidases are sperm adhesion molecule1 (SPAM1), also known as PH-20, which is predominantly expressed in germ cells in mice. SPAM1 is a 52-kDa membrane protein expressed on the surface of sperms. It consists of the following three domains: a hyaluronidase domain, a ZP-binding domain, and a glycosylphosphatidylinositol (GPI)-anchor domain [7, 8]. It was first discovered in guinea pig by the Myles group and later reported in many other species [9-11]. Furthermore, until the discovery of testicular hyaluronidases, SPAM1 was considered the only sperm factor involved in fertilization [8]. In guinea pigs, it was found to localize to the sperm membrane and the posterior head surface and move to the inner acrosomal membrane after the acrosome reaction [9]. SPAM1 is reported to be involved the first interaction between ejaculated sperms and an oocyte ovulated at the ampullae osseae tubae uterinae, which results in the dispersion of the cumulus cell mass surrounding the oocyte.

However, the expression pattern of SPAM1 in tissues is a bit controversial, largely because of the lack of agreement between the results of RT-PCR and Western blot. RT-PCR results suggest it to be expressed in prostate, placenta and the testis although Western blot analysis indicated that SPAM1 was expressed specifically in the testis [12]. Furthermore, IVF inhibition assay using recombinant protein and antibody against SPAM1 revealed that it is involved in the decomposition of the cumulus mass and the binding of sperm with the ZP surrounding the egg [12]. Many researchers believed that male SPAM1 knock-out mouse would be infertile. However, a female mouse mated with the knock-out male mouse produced offspring normally, except for the gestation period being longer by one day [13]. Thus, contrary to expectations, SPAM1 was confirmed to be inessential for fertilization in mice. Kim et al. [14] discovered a new hyaluronidase, Hyal5, in the sperm of SPAM1 knock-out mouse. Hyal5 was found to have several similarities with SPAM1.

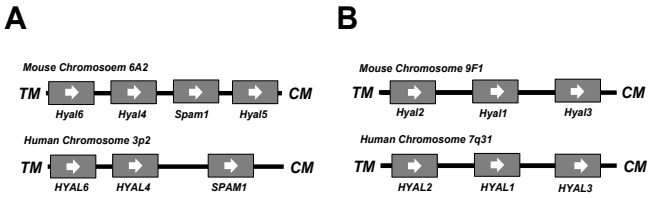

Figure 1. The chromosomal orientation of the six hyaluronidase gene clusters between mouse and human. A. Somatic hyaluronidases. B. Reproductive hyaluronidases. TM, telomere; CM, centromere. Closed boxes represent hyaluronidase genes on the genomic region.

The Hyal5 gene was found to be next to the SPAM1 gene on chromosome 6 [14]. Hyal5 is a 55-kDa protein, slightly larger than the 52-kDa SPAM1. The expression of Hyal5 is specifically restricted from testicular germ cells to ejaculated sperm in mice, and it is thought to contain a GPI anchor protein that includes hyaluronidase and ZP-binding domains [14]. SPAM1 and Hyal5 exhibit many parallel traits, including similar amino acid sequences, chromosomal location, and protein expression patterns [15]. However, unlike SPAM1, which exists on the surface of sperm, Hyal5 exists on the surface of sperm as well as the outer acrosomal membrane, suggesting that the two hyaluronidases may play different roles during fertilization. In addition, the hyaluronidase activity of Hyal5 is much higher than that of SPAM1 [14]. Although essential for germ cell-specific hyaluronidase functions, the precise roles of SPAM1 and Hyal5 remain unclear. Hyal5 is also different from mouse SPAM1 in several aspects. *Hyal5* only exists in rodents, while *PH-20* exists in all mammals. Hyal5 has cysteine common to sperm hyaluronidase. However, it has more glutamic acid, which is a potential sugar chain-binding site. Zymography analysis showed that SPAM1 of large animals such as pigs, bulls, and horses has a very high activation [16], unlike the hyaluronidase of rodents such as mouse. Mouse SPAM1 is known to be a double-chain protein that can be cut inside by a site called Arg^{346}–Ala^{347}connected with covalently linked disulfide bridges. However, the potential cutting site is replaced by Thr^{347}-Met^{348} in Hyal5 [14]. Therefore, Hyal5 is different from SPAM1structurally, thus influencing the degree of activation.

Hyal5 knock-out male mouse was generated by Kimura et al. in 2009 [17]. Unexpectedly, although the ability to disperse the cumulus cell mass was reduced, these mice carrying a null mutation in the Hyal5 gene, were fertile. Experiments on knock-out mice showed that SPAM1 and Hyal5 function complementarily.

Conclusion

Many earlier studies suggested that SPAM1 is the only hyaluronidase in mammalian sperm, including that of guinea pigs, rats, macaques, humans, pig, and bull. In addition, although SPAM1 was thought to be essential for sperm penetration and dispersal of the cumulus cell mass surrounding the ovulated oocyte, sperm of SPAM1 knock-out mouse could penetrate the cumulus matrix, although the dispersal was delayed. Hyal5 is a newly identified rodent-specific sperm hyaluronidase, and Hyal5-deficient epididymal sperm were functionally normal. Therefore, no conclusion can be made on whether sperm hyaluronidase is essential for fertilization in mammals. To confirm their role, experiments should be conducted in male double-knockout mice lacking both genes.

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